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[Continued on next page]

(54) Title: CONJUGATES OF GLP-1 AGONISTS AND USES THEREOF

(57) Abstract: The present invention features a compound having the formula A-X-B, where A is peptide vector capable of enhancing transport of the compound across the blood-brain barrier or into particular cell types, X is a linker, and B is a GLP-1 agonist (e.g., exendin-4 or an exendin-4 analog). The compounds of the invention can be used to treat any disease where increased GLP-1 activity is desired, for example, metabolic diseases, such as obesity and diabetes.

Peptides	Amino acid sequence
Exendin-4 native	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS
Exendin-4-Lys(MHA)	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPK-(MHA)
(Cys32)-Exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPCSGAPPPS

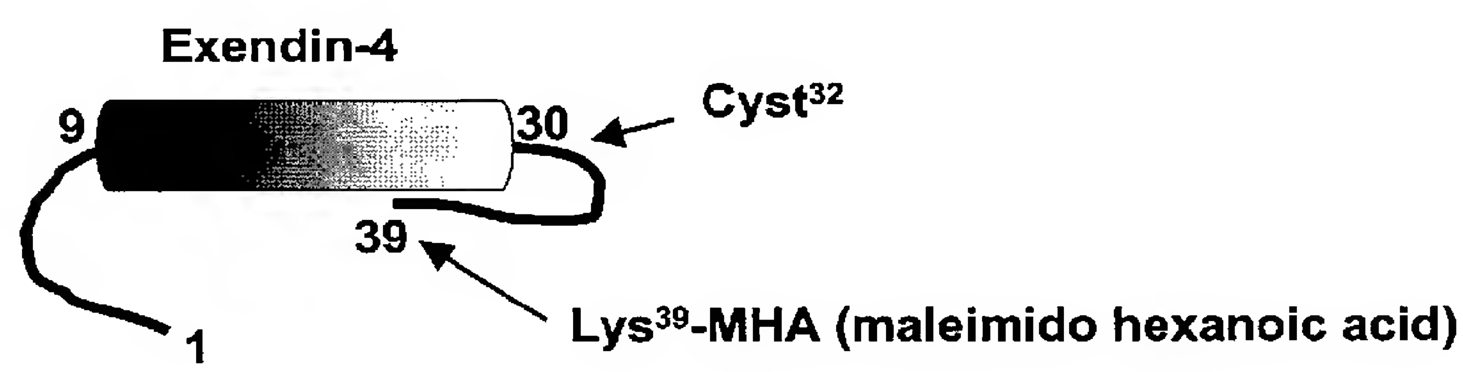

Figure 1

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CONJUGATES OF GLP-1 AGONISTS AND USES THEREOF

Background of the Invention

The invention relates to compounds including a GLP-1 agonist (e.g., exendin-4), bound to a peptide vector and uses thereof. Such uses include the treatment, prevention, and reduction of metabolic disorders including diabetes and obesity.

As the levels of blood glucose rise postprandially, insulin is secreted and stimulates cells of the peripheral tissues (skeletal muscles and fat) to actively take up glucose from the blood as a source of energy. Loss of glucose homeostasis as a result of faulty insulin secretion or action typically results in metabolic disorders such as diabetes, which may be co-triggered or further exacerbated by obesity. Because these conditions are often fatal, strategies to restore adequate glucose clearance from the bloodstream are required.

Although diabetes may arise secondary to any condition that causes extensive damage to the pancreas (e.g., pancreatitis, tumors, administration of certain drugs such as corticosteroids or pentamidine, iron overload (e.g., hemochromatosis), acquired or genetic endocrinopathies, and surgical excision), the most common forms of diabetes typically arise from primary disorders of the insulin signaling system. There are two major types of diabetes, namely type 1 diabetes (also known as insulin dependent diabetes (IDDM)) and type 2 diabetes (also known as insulin independent or non-insulin dependent diabetes (NIDDM)), which share common long-term complications in spite of their different pathogenic mechanisms.

Type 1 diabetes, which accounts for approximately 10% of all cases of primary diabetes, is an organ-specific autoimmune disease characterized by the extensive destruction of the insulin-producing beta cells of the pancreas. The consequent reduction in insulin production inevitably leads to the deregulation

of glucose metabolism. While the administration of insulin provides significant benefits to patients suffering from this condition, the short serum half-life of insulin is a major impediment to the maintenance of normoglycemia. An alternative treatment is islet transplantation, but this strategy has been associated with limited success.

Type 2 diabetes, which affects a larger proportion of the population, is characterized by a deregulation in the secretion of insulin and/or a decreased response of peripheral tissues to insulin, i.e., insulin resistance. While the pathogenesis of type 2 diabetes remains unclear, epidemiologic studies suggest that this form of diabetes results from a collection of multiple genetic defects or polymorphisms, each contributing its own predisposing risks and modified by environmental factors, including excess weight, diet, inactivity, drugs, and excess alcohol consumption. Although various therapeutic treatments are available for the management of type 2 diabetes, they are associated with various debilitating side effects. Accordingly, patients diagnosed with or at risk of having type 2 diabetes are often advised to adopt a healthier lifestyle, including loss of weight, change in diet, exercise, and moderate alcohol intake. Such lifestyle changes, however, are not sufficient to reverse the vascular and organ damages caused by diabetes.

Given that the strategies currently available for the management of metabolic disorders such as diabetes are suboptimal, there is a compelling need for treatments that are more effective and are not associated with such debilitating side effects.

Summary of the Invention

We have developed compounds that include a GLP-1 agonist (e.g., exendin-4) and a peptide vector. These compounds are useful in treating metabolic disorders such as diabetes and obesity. The peptide vector is capable of transporting the GLP-1 agonist either across the blood-brain barrier (BBB) or into a particular cell type (e.g., liver, lung, kidney, spleen, and muscle).

Because the conjugates are targeted across the BBB or to particular cell types, therapeutic efficacy can be achieved using lower doses or less frequent dosings as compared to unconjugated GLP-1 agonists, thus reducing the severity of or incidence of side effects and/or increasing efficacy. The conjugate may also exhibit increased stability, improved pharmacokinetics, or reduced degradation in vivo.

Accordingly, in a first aspect the invention features a compound having the formula:

A-X-B

where A is a peptide vector capable of being transported across the blood-brain barrier (BBB) or into a particular cell type (e.g., liver, lung, kidney, spleen, and muscle), X is a linker, and B is a GLP-1 agonist (e.g., any described herein such as a peptide agonist). The transport across the BBB or into the cell may be increased by at least 10%, 25%, 50%, 75%, 100%, 200%, 500%, 750%, 1000%, 1500%, 2000%, 5000%, or 10,000%. The compound may be substantially pure. The compound may be formulated with a pharmaceutically acceptable carrier (e.g., any described herein).

In another aspect, the invention features methods of making the compound A-X-B. In one embodiment, the method includes conjugating the peptide vector (A) to a linker (X), and conjugating the peptide vector-linker (A-X) to a GLP-1 agonist (B), thereby forming the compound A-X-B. In another embodiment, the method includes conjugating the GLP-1 agonist (B) to a linker (X), and conjugating the GLP-1 agonist/linker (X-B) to a peptide vector (A), thereby forming the compound A-X-B. In another embodiment, the method includes conjugating the peptide vector (A) to a GLP-1 agonist (B), where either A or B optionally include a linker (X), to form the compound A-X-B.

In another aspect, the invention features a nucleic acid molecule that encodes the compound A-X-B, where the compound is a polypeptide. The nucleic acid molecule may be operably linked to a promoter and may be part of a nucleic acid vector. The vector may be in a cell, such as a prokaryotic cell

(e.g., bacterial cell) or eukaryotic cell (e.g., yeast or mammalian cell, such as a human cell).

In another aspect, the invention features methods of making a compound of the formula A-X-B, where A-X-B is a polypeptide. In one embodiment, the method includes expressing a nucleic acid vector of the previous aspect in a cell to produce the polypeptide; and purifying the polypeptide.

In another aspect, the invention features a method of treating (e.g., prophylactically) a subject having a metabolic disorder. The method includes administering a compound of the first aspect in an amount sufficient to treat the disorder. The metabolic disorder may be diabetes (e.g., Type I or Type II), obesity, diabetes as a consequence of obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, impaired glucose tolerance (IGT), diabetic dyslipidemia, hyperlipidemia, a cardiovascular disease, or hypertension.

In another aspect, the invention features a method of reducing food intake by, or reducing body weight of, a subject. The method includes administering a compound of the first aspect to a subject in an amount sufficient to reduce food intake or reduce body weight. The subject may be overweight, obese, or bulimic.

In another aspect, the invention features a method of treating (e.g., prophylactically) a disorder selected from the group consisting of anxiety, movement disorder, aggression, psychosis, seizures, panic attacks, hysteria, sleep disorders, Alzheimer's disease, and Parkinson's disease. The method includes administering a compound of the first aspect to a subject in an amount sufficient to treat or prevent the disorder.

The invention also features a method of increasing neurogenesis in a subject. The method includes administering a compound of the first aspect to a subject. The subject may desire, or may be in need of neurogenesis. In certain embodiments, the subject may be suffering from a disease or disorder of the central nervous system such as Parkinson's Disease, Alzheimer's Disease,

Huntington's Disease, ALS, stroke, ADD, and neuropsychiatric syndromes. In other embodiments, the increase in neurogenesis can improve learning or enhance neuroprotection.

In another aspect, the invention features a method for converting liver stem/progenitor cells into functional pancreatic cells; preventing beta-cell deterioration and stimulation of beta-cell proliferation; treating obesity; suppressing appetite and inducing satiety; treating irritable bowel syndrome; reducing the morbidity and/or mortality associated with myocardial infarction and stroke; treating acute coronary syndrome characterized by an absence of Q-wave myocardial infarction; attenuating post-surgical catabolic changes; treating hibernating myocardium or diabetic cardiomyopathy; suppressing plasma blood levels of norepinepherine; increasing urinary sodium excretion, decreasing urinary potassium concentration; treating conditions or disorders associated with toxic hypervolemia, e.g., renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, and hypertension; inducing an inotropic response and increasing cardiac contractility; treating polycystic ovary syndrome; treating respiratory distress; improving nutrition via a non-alimentary route, i.e., via intravenous, subcutaneous, intramuscular, peritoneal, or other injection or infusion; treating nephropathy; treating left ventricular systolic dysfunction (e.g., with abnormal left ventricular ejection fraction); inhibiting antro-duodenal motility (e.g., for the treatment or prevention of gastrointestinal disorders such as diarrhea, postoperative dumping syndrome and irritable bowel syndrome, and as premedication in endoscopic procedures ; treating critical illness polyneuropathy (CIPN) and systemic inflammatory response syndrome (SIRS; modulating triglyceride levels and treating dyslipidemia; treating organ tissue injury caused by reperfusion of blood flow following ischemia; or treating coronary heart disease risk factor (CHDRF) syndrome in a subject by administering and effective amount of a GLP-1 agonist.

In another aspect, the invention features a method of increasing GLP-1 receptor activity in a subject. The method includes administering a compound of the first aspect to a subject in an amount sufficient to increase GLP-1 receptor activity. The method may reduce glucose levels in a subject.

In any of the methods involving administration of a compound to a subject, the amount sufficient may be less than 90%, 75%, 50%, 40%, 30%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or 0.1% of the amount required for an equivalent dose of the GLP-1 agonist when not conjugated to the peptide vector. The amount sufficient may reduce side effects (e.g., vomiting, nausea, or diarrhea) as compared to administration of an effective amount of the GLP-1 agonist when not conjugated to the peptide vector. The subject may be a mammal such as a human.

In any of the above aspects, the peptide vector may be a polypeptide substantially identical to any of the sequences set Table 1, or a fragment thereof. In certain embodiments, the vector polypeptide has a sequence of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), Angiopep-3 (SEQ ID NO:107), Angiopep-4a (SEQ ID NO:108), Angiopep-4b (SEQ ID NO:109), Angiopep-5 (SEQ ID NO:110), Angiopep-6 (SEQ ID NO:111), or Angiopep-7 (SEQ ID NO:112)). The peptide vector or conjugate may be efficiently transported into a particular cell type (e.g., any one, two, three, four, or five of liver, lung, kidney, spleen, and muscle) or may cross the mammalian BBB efficiently (e.g., Angiopep-1, -2, -3, -4a, -4b, -5, and -6). In another embodiment, the peptide vector or conjugate is able to enter a particular cell type (e.g., any one, two, three, four, or five of liver, lung, kidney, spleen, and muscle) but does not cross the BBB efficiently (e.g., a conjugate including Angiopep-7). The peptide vector may be of any length, for example, at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 35, 50, 75, 100, 200, or 500 amino acids, or any range between these numbers. In certain embodiments, the peptide vector is 10 to 50 amino acids in length. The polypeptide may be produced by recombinant genetic technology or chemical synthesis.

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Table 1: Exemplary Polypeptides

SEQ ID NO:																			
1	T	F	V	Y	G	G	C	R	A	K	R	N	N	F	K	S	A	E	D
2	T	F	Q	Y	G	G	C	M	G	N	G	N	N	F	V	T	E	K	E
3	P	F	F	Y	G	G	C	G	G	N	R	N	N	F	D	T	E	E	Y
4	S	F	Y	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	E
5	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
6	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	Y
7	T	F	F	Y	G	G	C	R	A	K	K	N	N	Y	K	R	A	K	Y
8	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
9	T	F	Q	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
10	T	F	Q	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
11	T	F	F	Y	G	G	C	L	G	K	R	N	N	F	K	R	A	K	Y
12	T	F	F	Y	G	G	S	L	G	K	R	N	N	F	K	R	A	K	Y
13	P	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	R	A	K	Y
14	T	F	F	Y	G	G	C	R	G	K	G	N	N	Y	K	R	A	K	Y
15	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	A	K	Y
16	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	E	K	Y
17	P	F	F	Y	G	G	C	R	A	K	K	N	N	F	K	R	A	K	E
18	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	D
19	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	D	R	A	K	Y
20	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	E	Y
21	P	F	F	Y	G	G	C	G	A	N	R	N	N	F	K	R	A	K	Y
22	T	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	T	A	K	Y
23	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	R	A	K	Y
24	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	A	K	Y
25	T	F	F	Y	G	G	S	R	G	N	R	N	N	F	K	T	A	K	Y
26	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	K	R	A	K	Y
27	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	A	K	Y
28	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	A	K	Y
29	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	K	Y
30	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	D	R	E	K	Y
31	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	E	K	E
32	T	F	F	Y	G	G	C	R	G	K	G	N	N	F	D	R	A	K	Y
33	T	F	F	Y	G	G	S	R	G	K	G	N	N	F	D	R	A	K	Y

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34	T F F Y G G C R G N G N N F V T A K Y
35	P F F Y G G C G G K G N N Y V T A K Y
36	T F F Y G G C L G K G N N F L T A K Y
37	S F F Y G G C L G N K N N F L T A K Y
38	T F F Y G G C G G N K N N F V R E K Y
39	T F F Y G G C M G N K N N F V R E K Y
40	T F F Y G G S M G N K N N F V R E K Y
41	P F F Y G G C L G N R N N Y V R E K Y
42	T F F Y G G C L G N R N N F V R E K Y
43	T F F Y G G C L G N K N N Y V R E K Y
44	T F F Y G G C G G N G N N F L T A K Y
45	T F F Y G G C R G N R N N F L T A E Y
46	T F F Y G G C R G N G N N F K S A E Y
47	P F F Y G G C L G N K N N F K T A E Y
48	T F F Y G G C R G N R N N F K T E E Y
49	T F F Y G G C R G K R N N F K T E E D
50	P F F Y G G C G G N G N N F V R E K Y
51	S F F Y G G C M G N G N N F V R E K Y
52	P F F Y G G C G G N G N N F L R E K Y
53	T F F Y G G C L G N G N N F V R E K Y
54	S F F Y G G C L G N G N N Y L R E K Y
55	T F F Y G G S L G N G N N F V R E K Y
56	T F F Y G G C R G N G N N F V T A E Y
57	T F F Y G G C L G K G N N F V S A E Y
58	T F F Y G G C L G N R N N F D R A E Y
59	T F F Y G G C L G N R N N F L R E E Y
60	T F F Y G G C L G N K N N Y L R E E Y
61	P F F Y G G C G G N R N N Y L R E E Y
62	P F F Y G G S G G N R N N Y L R E E Y
63	M R P D F C L E P P Y T G P C V A R I
64	A R I I R Y F Y N A K A G L C Q T F V Y G
65	Y G G C R A K R N N Y K S A E D C M R T C G
66	P D F C L E P P Y T G P C V A R I I R Y F Y
67	T F F Y G G C R G K R N N F K T E E Y
68	K F F Y G G C R G K R N N F K T E E Y
69	T F Y Y G G C R G K R N N Y K T E E Y

70	T F F Y G G S R G K R N N F K T E E Y
71	C T F F Y G C C R G K R N N F K T E E Y
72	T F F Y G G C R G K R N N F K T E E Y C
73	C T F F Y G S C R G K R N N F K T E E Y
74	T F F Y G G S R G K R N N F K T E E Y C
75	P F F Y G G C R G K R N N F K T E E Y
76	T F F Y G G C R G K R N N F K T K E Y
77	T F F Y G G K R G K R N N F K T E E Y
78	T F F Y G G C R G K R N N F K T K R Y
79	T F F Y G G K R G K R N N F K T A E Y
80	T F F Y G G K R G K R N N F K T A G Y
81	T F F Y G G K R G K R N N F K R E K Y
82	T F F Y G G K R G K R N N F K R A K Y
83	T F F Y G G C L G N R N N F K T E E Y
84	T F F Y G C G R G K R N N F K T E E Y
85	T F F Y G G R C G K R N N F K T E E Y
86	T F F Y G G C L G N G N N F D T E E E
87	T F Q Y G G C R G K R N N F K T E E Y
88	Y N K E F G T F N T K G C E R G Y R F
89	R F K Y G G C L G N M N N F E T L E E
90	R F K Y G G C L G N K N N F L R L K Y
91	R F K Y G G C L G N K N N Y L R L K Y
92	K T K R K R K K Q R V K I A Y E E I F K N Y
93	K T K R K R K K Q R V K I A Y
94	R G G R L S Y S R R F S T S T G R
95	R R L S Y S R R R F
96	R Q I K I W F Q N R R M K W K K
97	T F F Y G G S R G K R N N F K T E E Y
98	M R P D F C L E P P Y T G P C V A R I I R Y F Y N A K A G L C Q T F V Y G G C R A K R N N F K S A E D C M R T C G G A
99	T F F Y G G C R G K R N N F K T K E Y
100	R F K Y G G C L G N K N N Y L R L K Y
101	T F F Y G G C R A K R N N F K R A K Y
102	N A K A G L C Q T F V Y G G C L A K R N N F

	E S A E D C M R T C G G A
103	Y G G C R A K R N N F K S A E D C M R T C G G A
104	G L C Q T F V Y G G C R A K R N N F K S A E
105	L C Q T F V Y G G C E A K R N N F K S A
107	T F F Y G G S R G K R N N F K T E E Y
108	R F F Y G G S R G K R N N F K T E E Y
109	R F F Y G G S R G K R N N F K T E E Y
110	R F F Y G G S R G K R N N F R T E E Y
111	T F F Y G G S R G K R N N F R T E E Y
112	T F F Y G G S R G R R N N F R T E E Y
113	C T F F Y G G S R G K R N N F K T E E Y
114	T F F Y G G S R G K R N N F K T E E Y C
115	C T F F Y G G S R G R R N N F R T E E Y
116	T F F Y G G S R G R R N N F R T E E Y C

Polypeptides Nos. 5, 67, 76, and 91, include the sequences of SEQ ID NOS:5, 67, 76, and 91, respectively, and are amidated at the C-terminus.
Polypeptides Nos. 107, 109, and 110 include the sequences of SEQ ID NOS:97, 109, and 110, respectively, and are acetylated at the N-terminus.

In any of the above aspects, the peptide vector may include an amino acid sequence having the formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19

where each of X1-X19 (e.g., X1-X6, X8, X9, X11-X14, and X16-X19) is, independently, any amino acid (e.g., a naturally occurring amino acid such as Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) or absent and at least one (e.g., 2 or 3) of X1, X10, and X15 is arginine. In some embodiments, X7 is Ser or Cys; or X10 and X15 each are independently Arg or Lys. In some embodiments, the residues from X1 through X19, inclusive, are substantially identical to any of the amino acid sequences of any one of SEQ ID NOS:1-105 and 107-116 (e.g., Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-

6, and Angiopep-7). In some embodiments, at least one (e.g., 2, 3, 4, or 5) of the amino acids X1-X19 is Arg. In some embodiments, the polypeptide has one or more additional cysteine residues at the N-terminal of the polypeptide, the C-terminal of the polypeptide, or both.

In any of the above aspects, the GLP-1 agonist may be a peptide agonist. The GLP-1 agonist may GLP-1, exendin-4, exendin-3, or analog or fragment thereof (e.g., any analog or fragment described herein). In particular embodiments, the GLP-1 agonist is an exendin-4 analog selected from the group consisting of [Lys^{39}]exendin-4 and [Cys^{32}]exendin-4.

In certain embodiments of any of the above aspects, the peptide vector or peptide GLP-1 agonist is modified (e.g., as described herein). The polypeptide may be amidated, acetylated, or both. Such modifications to polypeptides may be at the amino or carboxy terminus of the polypeptide. The polypeptide may also include peptidomimetics (e.g., those described herein) of any of the polypeptides described herein. The polypeptide may be in a multimeric form, for example, dimeric form (e.g., formed by disulfide bonding through cysteine residues).

In certain embodiments, the polypeptide has an amino acid sequence described herein with at least one amino acid substitution (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 substitutions), insertion, or deletion. The polypeptide may contain, for example, 1 to 12, 1 to 10, 1 to 5, or 1 to 3 amino acid substitutions, for example, 1 to 10 (e.g., to 9, 8, 7, 6, 5, 4, 3, 2) amino acid substitutions. The amino acid substitution(s) may be conservative or non-conservative. For example, the peptide vector may have an arginine at one, two, or three of the positions corresponding to positions 1, 10, and 15 of the amino acid sequence of any of SEQ ID NO:1, Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, and Angiopep-7. The GLP-1 agonist may have a cysteine or lysine substitution or addition at any position (e.g., a lysine substitution at the N- or C-terminal position, or a cysteine substitution at the position corresponding to amino acid 32 of the exendin-4 sequence).

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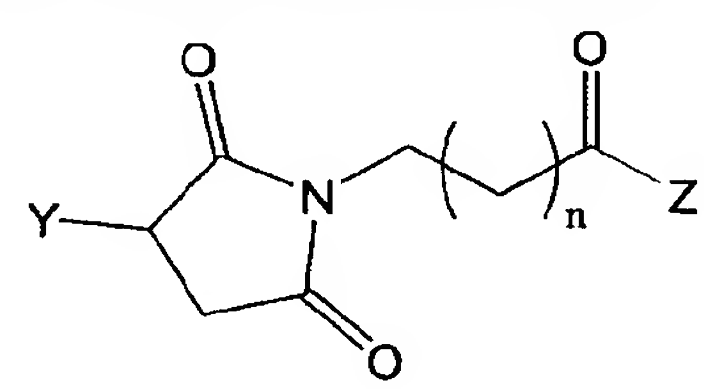
In any of the above aspects, the compound may specifically exclude a polypeptide including or consisting of any of SEQ ID NOS:1-105 and 107-116 (e.g., Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, and Angiopep-7). In some embodiments, the polypeptides and conjugates of the invention exclude the polypeptides of SEQ ID NOs:102, 103, 104, and 105.

In any of the above aspects, the linker (X) may be any linker known in the art or described herein. In particular embodiments, the linker is a covalent bond (e.g., a peptide bond), a chemical linking agent (e.g., those described herein), an amino acid or a peptide (e.g., 2, 3, 4, 5, 8, 10, or more amino acids). In certain embodiments, the linker has the formula:



where n is an integer between 2 and 15 (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15); and either Y is a thiol on A and Z is a primary amine on B or Y is a thiol on B and Z is a primary amino on A.

By “GLP-1 agonist” is meant any compound capable of activating a GLP-1 receptor (e.g., a mammalian or human GLP-1 receptor). Agonists can include peptides or small molecule compounds (e.g., any of those described herein). Assays for determining whether a particular compound is a GLP-1 agonist are known in the art and described herein.

By “peptide vector” is meant a compound or molecule such as a polypeptide or a polypeptide mimetic that can be transported into a particular cell type (e.g., liver, lungs, kidney, spleen, or muscle) or across the BBB. The vector may be attached to (covalently or not) or conjugated to an agent (e.g., a GLP-1 agonist) and thereby may be able to transport the agent into a particular cell type or across the BBB. In certain embodiments, the vector may bind to receptors present on cancer cells or brain endothelial cells and thereby be

transported into the cancer cell or across the BBB by transcytosis. The vector may be a molecule for which high levels of transendothelial transport may be obtained, without affecting the cell or BBB integrity. The vector may be a polypeptide or a peptidomimetic and may be naturally occurring or produced by chemical synthesis or recombinant genetic technology.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95%, or even 99% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, or 100) amino acids. For nucleic acids, the length of comparison sequences will generally be at least 60 nucleotides, preferably at least 90 nucleotides, and more preferably at least 120 nucleotides, or full length. It is to be understood herein that gaps may be found between the amino acids of an analogs that are identical or similar to amino acids of the original polypeptide. The gaps may include no amino acids, one or more amino acids that are not identical or similar to the original polypeptide. Biologically active analogs of the vectors (polypeptides) of the invention are encompassed herewith. Percent identity may be determined, for example, with n algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

By "treating" a disease, disorder, or condition in a subject is meant reducing at least one symptom of the disease, disorder, or condition by administrating a therapeutic agent to the subject.

By "treating prophylactically" a disease, disorder, or condition in a subject is meant reducing the frequency of occurrence of (e.g., preventing) a disease, disorder or condition or reducing the severity of the disease, disorder, or condition by administering a therapeutic agent to the subject.

A subject who is being treated for a metabolic disorder is one who a medical practitioner has diagnosed as having such a condition. Diagnosis may be performed by any suitable means, such as those described herein. A subject

in whom the development of diabetes or obesity is being prevented may or may not have received such a diagnosis. One in the art will understand that subject of the invention may have been subjected to standard tests or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors, such as family history, obesity, particular ethnicity (e.g., African Americans and Hispanic Americans), gestational diabetes or delivering a baby that weighs more than nine pounds, hypertension, having a pathological condition predisposing to obesity or diabetes, high blood levels of triglycerides, high blood levels of cholesterol, presence of molecular markers (e.g., presence of autoantibodies), and age (over 45 years of age). An individual is considered obese when their weight is 20% (25% in women) or more over the maximum weight desirable for their height. An adult who is more than 100 pounds overweight, is considered to be morbidly obese. Obesity is also defined as a body mass index (BMI) over 30 kg/m^2.

By "a metabolic disorder" is meant any pathological condition resulting from an alteration in a subject's metabolism. Such disorders include those resulting from an alteration in glucose homeostasis resulting, for example, in hyperglycemia. According to this invention, an alteration in glucose levels is typically an increase in glucose levels by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% relative to such levels in a healthy individual. Metabolic disorders include obesity and diabetes (e.g., diabetes type I, diabetes type II, MODY, and gestational diabetes), satiety, and endocrine deficiencies of aging.

By "reducing glucose levels" is meant reducing the level of glucose by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% relative to an untreated control. Desirably, glucose levels are reduced to normoglycemic levels, i.e., between 150 to 60 mg/dL, between 140 to 70 mg/dL, between 130 to 70 mg/dL, between 125 to 80 mg/dL, and preferably between 120 to 80 mg/dL. Such reduction in glucose levels may be obtained by

increasing any one of the biological activities associated with the clearance of glucose from the blood (e.g., increase insulin production, secretion, or action).

By "subject" is meant a human or non-human animal (e.g., a mammal).

By "increasing GLP-1 receptor activity" is meant increasing the level of receptor activation measured using standard techniques (e.g., cAMP activation) by, for example, at least %, 20%, 50%, 75%, 100%, 200%, or 500% as compared to an untreated control.

By "equivalent dosage" is meant the amount of a compound of the invention required to achieve the same molar amount of the GLP-1 agonist in the compound, as compared to the unconjugated GLP-1 agonist. For example, the equivalent dosage of 1.0 μg exendin-4 is about 1.6 μg of the [Lys^{39}-MHA]exendin-4/Angiopep-2-Cys-NH_2 conjugate described herein.

By a polypeptide which is "efficiently transported across the BBB" is meant a polypeptide that is able to cross the BBB at least as efficiently as Angiopep-6 (i.e., greater than 38.5% that of Angiopep-1 (250 nM) in the *in situ* brain perfusion assay described in U.S. Patent Application No. 11/807,597, filed May 29, 2007, hereby incorporated by reference). Accordingly, a polypeptide which is "not efficiently transported across the BBB" is transported to the brain at lower levels (e.g., transported less efficiently than Angiopep-6).

By a polypeptide or compound which is "efficiently transported to a particular cell type" is meant that the polypeptide or compound is able to accumulate (e.g., either due to increased transport into the cell, decreased efflux from the cell, or a combination thereof) in that cell type to at least a 10% (e.g., 25%, 50%, 100%, 200%, 500%, 1,000%, 5,000%, or 10,000%) greater extent than either a control substance, or, in the case of a conjugate, as compared to the unconjugated agent. Such activities are described in detail in International Application Publication No. WO 2007/009229, hereby incorporated by reference.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figure 1 is table and schematic diagram showing exendin-4 and the exendin-4 analogs used in experiments described herein.

Figure 2 is a schematic diagram of the synthetic scheme used to conjugate Cys-AngioPep2, Angiopep-2-Cys-NH_2, and Angiopep-1 to [Lys^{39}-MHA]exendin-4.

Figure 3 is a schematic diagram of the synthetic scheme used to conjugate [Cys^{32}]exendin-4 to (maleimido propionic acid (MPA))-Angiopep-2, (maleimido hexamoic acid (MHA))-Angiopep-2, and (maleimido undecanoic acid (MUA))-Angiopep-2.

Figure 4 is a graph showing transport of exendin-4 (left) and exendin-4/Angiopep-2 (N-terminal, center; c-terminal, right) across the BBB. The total amount in the brain, along with the amounts in the capillaries and the parenchyma are shown.

Figure 5 is a graph showing increase in weight of (ob/ob) mice following administration of a control, exendin-4, or the [Lys^{39}-MHA]exendin-4/Angiopep-2-Cys-NH_2 conjugate (Exen-An2). Both exendin-4 and Ex-An2 were observed to reduce weight gain as compared to the animals receiving the control.

Figure 6 is a graph showing total food consumption by (ob/ob) mice, where the mice were administered a control, exendin-4, or the Exen-An2. Both exendin-4 and Exen-An2 were observed to reduce food intake as compared to the animals receiving the control.

Figure 7 is a graph showing reduction in glycemia following administration of two doses of exendin-4 (3 μg/kg and 30 μg/kg) and equivalent doses of Exen-An2 (4.8 μg/kg and 48 μg/kg). A similar reduction in glycemia at the lower dose of Exen-An2, as compared to the higher dose of exendin-4, was observed. During this experiment, one mouse in the control group died at day 12.

Figure 8A is a schematic diagram showing the structure of an Exendin-4-Angiopep-2 dimer conjugate (Ex4(Lys39(MHA))-AN2-AN2). The compound has the structure HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPK(MHA)-TFFYGGSRGKRNNFKTEEYC-(MPA)-TFFYGGSRGKRNNFKTEEY-OH, where MHA is maleimido hexanoic acid and MPA is maleimido propionic acid.

Figure 8B is a schematic structure of an Exendin-4-scramble-Angiopep-2 (Ex4(Cys32)-ANS4 (N-Term) or Exen-S4) that was used a control. This compound has the structure HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPCSGAPPPS-(MHA)-GYKGERYRGFKETNFNTFS-OH, where MHA is maleimido hexanoic acid.

Figure 9 is a graph showing the ability of, from left to right, Exendin-4; Exendin-4-Angiopep-2 conjugates C3, C6, and C11; Exen-S4; and Exendin-4 when conjugated to a dimeric form of Angiopep-2, to cross the BBB.

Figure 10 is a graph showing the ability of Exendin-4 and Exen-An2-An2 to reduce glycemia in mice.

Detailed Description

We have developed GLP-1 agonist/peptide conjugates having an enhanced ability to cross the blood-brain barrier (BBB) or to enter particular cell type(s) (e.g., liver, lung, kidney, spleen, and muscle) using the exemplary GLP-1 agonist exendin-4 and exendin-4 analogs. The peptide conjugates of the invention can include a GLP-1 agonist and a peptide vector that enhance transport across the BBB.

We have also shown that lower doses of the compounds of the invention, as compared to unconjugated GLP-1 agonists, are effective in treating GLP-1 related disorders including a reduction in glycemia. By administering lower doses of the conjugated peptides, side effects such as vomiting, nausea, and diarrhea observed with the unconjugated agonists can be reduced or eliminated. Alternatively, increased efficacy at higher doses may be obtained.

The GLP-1 agonist can be any GLP-1 agonist known in the art and including peptides such as those described below. Particular GLP-1 agonists include exendin-4, GLP-1, and exendin-3 fragments, substitutions (e.g., conservative or nonconservative substitutions, or substitutions of non-naturally occurring amino acids), and chemical modifications to the amino acid sequences (e.g., those described herein). Particular GLP-1 agonists are described in detail below.

GLP-1 agonists

The conjugates of the invention can include any GLP-1 agonist known in the art. Particular GLP-1 agonists include GLP-1, exendin-4, and analogs thereof. Exemplary analogs are described below.

Exendin-4 and exendin-4 analogs

Exendin-4 and exendin-4 analogs can also be used in the compositions, methods, and kits of the invention. The compounds of the invention can include fragments of the exendin-4 sequence. Exendin-4 has the sequence.

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2

Particular exendin-4 analogs include those having a cysteine substitution (e.g., [Cys^{32}]exendin-4) or a lysine substitution (e.g., [Lys^{39}]exendin-4).

Exendin analogs are also described in U.S. Patent No. 7,157,555 and include those of the formula:

X_1-X_2-X_3-Gly-Thr-X_4-X_5-X_6-X_7-X_8-Ser-Lys-Gln-X_9-Glu-Glu-Glu-Ala-Val-Arg-Leu-X_{10}-X_{11}-X_{12}-X_{13}-Leu-Lys-Asn-Gly-Gly-X_{14}-Ser-Ser-Gly-Ala-X_{15}-X_{16}-X_{17}-X_{18}-Z

where X_1 is His, Arg or Tyr; X_2 is Ser, Gly, Ala or Thr; X_3 is Asp or Glu; X_4 is Phe, Tyr or Nal; X_5 is Thr or Ser; X_6 is Ser or Thr; X_7 is Asp or Glu; X_8 is Leu, Ile, Val, pGly or Met; X_9 is Leu, Ile, pGly, Val or Met; X_{10} is Phe, Tyr, or Nal;

X_{11} is Ile, Val, Leu, pGly, t-BuG or Met; X_{12} is Glu or Asp; X_{13} is Trp, Phe, Tyr, or Nal; X_{14}, X_{15}, X_{16} and X_{17} are independently Pro, HPro, 3Hyp, 4Hyp, TPro, N-alkylglycine, N-alkyl-pGly, or N-alkylalanine; X_{18} is Ser, Thr, or Tyr; and Z is –OH or $-NH_2$ (e.g., with the proviso that the compound is not exendin-3 or exindin-4.)

Preferred N-alkyl groups for N-alkylglycine, N-alkyl-pGly and N-alkylalanine include lower alkyl groups (e.g., C_{1-6} alkyl or C_{1-4} alkyl).

In certain embodiments, X_1 is His or Tyr (e.g., His). X_2 can be Gly. X_9 can be Leu, pGly, or Met. X_{13} can be Trp or Phe. X_4 can be Phe or Nal; X_{11} can be Ile or Val, and X_{14}, X_{15}, X_{16} and X_{17} can be independently selected from Pro, HPro, TPro, or N-alkylalanine (e.g., where N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms). In one aspect, X_{15}, X_{16}, and X_{17} are the same amino acid residue. X_{18} may be Ser or Tyr (e.g., Ser). Z can be $-NH_2$.

In other embodiments, X_1 is His or Tyr (e.g., His); X_2 is Gly; X_4 is Phe or Nal; X_9 is Leu, pGly, or Met; X_{10} is Phe or Nal; X_{11} is Ile or Val; X_{14}, X_{15}, X_{16}, and X_{17} are independently selected from Pro, HPro, TPro, or N-alkylalanine; and X_{18} is Ser or Tyr, (e.g., Ser). Z can be $-NH_2$.

In other embodiments, X_1 is His or Arg; X_2 is Gly; X_3 is Asp or Glu; X_4 is Phe or napthylalanine; X_5 is Thr or Ser; X_6 is Ser or Thr; X_7 is Asp or Glu; X_8 is Leu or pGly; X_9 is Leu or pGly; X_{10} is Phe or Nal; X_{11} is Ile, Val, or t-butyltylglycine; X_{12} is Glu or Asp; X_{13} is Trp or Phe; X_{14}, X_{15}, X_{16}, and X_{17} are independently Pro, HPro, TPro, or N-methylalanine; X_{18} is Ser or Tyr: and Z is –OH or $-NH_2$ (e.g., where the compound is not exendin-3 or exendin-4). Z can be $-NH_2$.

In another embodiment, X_9 is Leu, Ile, Val, or pGly (e.g., Leu or pGly) and X_{13} is Phe, Tyr, or Nal (e.g., Phe or Nal). These compounds can exhibit advantageous duration of action and be less subject to oxidative degradation, both in vitro and in vivo, as well as during synthesis of the compound.

Other exendin analogs also described in U.S. Patent Nos. 7,157,555 and 7,223,725, include compounds of the formula:

X_1-X_2-X_3-Gly-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-Ala-X_{19}-X_{20}-X_{21}-X_{22}-X_{23}-X_{24}-X_{25}-X_{26}-X_{27}-X_{28}-Z_1

where X_1 is His, Arg, or Tyr; X_2 is Ser, Gly, Ala, or Thr; X_3 is Asp or Glu; X_5 is Ala or Thr; X_6 is Ala, Phe, Tyr, or Nal; X_7 is Thr or Ser; X_8 is Ala, Ser, or Thr; X_9 is Asp or Glu; X_{10} is Ala, Leu, Ile, Val, pGly, or Met; X_{11} is Ala or Ser; X_{12} is Ala or Lys; X_{13} is Ala or Gln; X_{14} is Ala, Leu, Ile, pGly, Val, or Met; X_{15} is Ala or Glu; X_{16} is Ala or Glu; X_{17} is Ala or Glu; X_{19} is Ala or Val; X_{20} is Ala or Arg; X_{21} is Ala or Leu; X_{22} is Phe, Tyr, or Nal; X_{23} is Ile, Val, Leu, pGly, t-BuG, or Met; X_{24} is Ala, Glu, or Asp; X_{25} is Ala, Trp, Phe, Tyr, or Nal; X_{26} is Ala or Leu; X_{27} is Ala or Lys; X_{28} is Ala or Asn; Z_1 is –OH, $-NH_2$, Gly-Z_2, Gly-Gly-Z_2, Gly-Gly-X_{31}-Z_2, Gly-Gly-X_{31}-Ser-Z_2, Gly-Gly-X_{31}-Ser-Ser-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{36}-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{36}-X_{37}-Z_2 or Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{36}-X_{37}-X_{38}-Z_2; X_{31}, X_{36}, X_{37}, and X_{38} are independently Pro, HPro, 3Hyp, 4Hyp, TPro, N-alkylglycine, N-alkyl-pGly or N-alkylalanine; and Z_2 is –OH or $-NH_2$ (e.g., provided that no more than three of X_5, X_6, X_8, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{19}, X_{20}, X_{21}, X_{24}, X_{25}, X_{26}, X_{27} and X_{28} are Ala). Preferred N-alkyl groups for N-alkylglycine, N-alkyl-pGly and N-alkylalanine include lower alkyl groups of 1 to about 6 carbon atoms (e.g., 1 to 4 carbon atoms).

In certain embodiments, X_1 is His or Tyr (e.g., His). X_2 can be Gly. X_{14} can be Leu, pGly, or Met. X_{25} can be Trp or Phe. In some embodiments, X_6 is Phe or Nal, X_{22} is Phe or Nal, and X_{23} is Ile or Val. X_{31}, X_{36}, X_{37}, and X_{38} can be independently selected from Pro, HPro, TPro, and N-alkylalanine. In certain embodiments, Z_1 is $-NH_2$ or Z_2 is $-NH_2$.

In another embodiment, X_1 is His or Tyr (e.g., His); X_2 is Gly; X_6 is Phe or Nal; X_{14} is Leu, pGly, or Met; X_{22} is Phe or Nal; X_{23} is Ile or Val; X_{31}, X_{36}, X_{37}, and X_{38} are independently selected from Pro, HPro, TPro, or N-alkylalanine. In particular embodiments, Z_1 is $-NH_2$.

In another embodiment, X_1 is His or Arg; X_2 is Gly or Ala; X_3 is Asp or Glu; X_5 is Ala or Thr; X_6 is Ala, Phe, or naphthylalanine; X_7 is Thr or Ser; X_8 is Ala, Ser, or Thr; X_9 is Asp or Glu; X_{10} is Ala, Leu, or pGly; X_{11} is Ala or Ser; X_{12} is Ala or Lys; X_{13} is Ala or Gln; X_{14} is Ala, Leu, or pGly; X_{15} is Ala or Glu; X_{16} is Ala or Glu; X_{17} is Ala or Glu; X_{19} is Ala or Val; X_{20} is Ala or Arg; X_{21} is Ala or Leu; X_{22} is Phe or Nal; X_{23} is Ile, Val or t-BuG; X_{24} is Ala, Glu or Asp; X_{25} is Ala, Trp or Phe; X_{26} is Ala or Leu; X_{27} is Ala or Lys; X_{28} is Ala or Asn; Z_1 is –OH, $-NH_2$, Gly-Z_2, Gly-Gly-Z_2, Gly-Gly-X_{31}-Z_2, Gly-Gly X_{31}-Ser-Z_2, Gly-Gly-X_{31} Ser-Ser-Z_2, Gly-Gly-X_{31} Ser-Ser-Gly-Z_2, Gly-Gly-X_{31} Ser-Ser-Gly Ala-Z_2, Gly-Gly-X_{31} Ser-Ser-Gly-Ala-X_{36}-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{36}-X_{37}-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{36}-X_{37}-X_{38}-Z_2; X_{31}, X_{36}, X_{37} and X_{38} being independently Pro HPro, TPro or N-methylalanine; and Z_2 being –OH or $-NH_2$ (e.g., provided that no more than three of X_3, X_5, X_6, X_8, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{19}, X_{20}, X_{21}, X_{24}, X_{25}, X_{26}, X_{27} and X_{28} are Ala).

In yet another embodiment, X_{14} is Leu, Ile, Val, or pGly (e.g., Leu or pGly), and X_{25} is Phe, Tyr or Nal (e.g., Phe or Nal).

Exendin analogs described in U.S. Patent No. 7,220,721 include compounds of the formula:

X_1-X_2-X_3-X_4-X_{-5}-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-Ala-X_{19}-X_{20}-X_{21}-X_{22}-X_{23}-X_{24}-X_{25}-X_{26}-X_{27}-X_{28}-Z_1

where X_1 is His, Arg, Tyr, Ala, Norval, Val, or Norleu; X_2 is Ser, Gly, Ala, or Thr; X_3 is Ala, Asp, or Glu; X_4 is Ala, Norval, Val, Norleu, or Gly; X_5 is Ala or Thr; X_6 is Phe, Tyr or Nal; X_7 is Thr or Ser; X_8 is Ala, Ser or Thr; X_9 is Ala, Norval, Val, Norleu, Asp, or Glu; X_{10} is Ala, Leu, Ile, Val, pGly, or Met; X_{11} is Ala or Ser; X_{12} is Ala or Lys; X_{13} is Ala or Gln; X_{14} is Ala, Leu, Ile, pGly, Val, or Met; X_{15} is Ala or Glu; X_{16} is Ala or Glu; X_{17} is Ala or Glu; X_{19} is Ala or Val; X_{20} is Ala or Arg; X_{21} is Ala or Leu; X_{22} is Phe, Tyr, or Nal; X_{23} is Ile, Val, Leu, pGly, t-BuG, or Met; X_{24} is Ala, Glu, or Asp; X_{25} is Ala, Trp, Phe, Tyr, or Nal; X_{26} is Ala or Leu; X_{27} is Ala or Lys; X_{28} is Ala or Asn; Z_1 is –OH,

–NH_2, Gly-Z_2, Gly-Gly-Z_2, Gly-Gly-X_{31}-Z_2, Gly-Gly-X_{31}-Ser-Z_2, Gly-Gly-X_{31}-Ser-Ser-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Z_2, Gly-Gly-X_{31} Ser-Ser-Gly-Ala-Z_2, Gly-Gly-X_{31}-Ser-Ser-Gly-Ala-X_{13}-Z_2, Gly-Gly-X_{31} Ser-Ser-Gly-Ala-X_{36}-X_{37}-Z_2, Gly-Gly X_{31} Ser Ser Gly Ala X_{36} X_{37} X_{31} -Z_2 or Gly Gly X_{31} Ser Ser Gly Ala X_{36} X_{37} X_{38} X_{39} -Z_2 ; where X_{31}, X_{36}, X_{37}, and X_{38} are independently Pro, HPro, 3Hyp, 4Hyp, TPro, N-alkylglycine, N-alkyl-pGly, or N-alkylalanine; and Z_2 is –OH or –NH_2 (e.g., provided that no more than three of X_3, X_4, X_5, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{19}, X_{20}, X_{21}, X_{24}, X_{25}, X_{26}, X_{27} and X_{28} are Ala and/or provided also that, if X_1 is His, Arg, or Tyr, then at least one of X_3, X_4 and X_9 is Ala).

Particular examples of exendin-4 analogs include exendin-4(1-30), exendin-4(1-30) amide, exendin-4(1-28) amide, [Leu^{14},Phe^{25}]exendin-4 amide, [Leu^{14},Phe^{25}]exendin-4(1-28) amide, and [Leu^{14},Ala^{22},Phe^{25}]exendin-4(1-28) amide.

U.S. Patent No. 7,329,646 describes exendin-4 analogs having the general formula:

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-X_{14}-Glu-Glu-Glu-Ala-Val-X_{20}-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-X_{40}.

where X_{14} is Arg, Leu, Ile, or Met; X_{20} is His, Arg, or Lys; X_{40} is Arg-OH, –OH, –NH_2 or Lys-OH. In certain embodiments, when X_{14} is Met and X_{20} is Arg, X_{40} cannot be –NH_2. Other exendin-4 derivatives include [(Ile/Leu/Met)^{14},(His/Lys)^{20},Arg^{40}]exendin-4; [(not Lys/not Arg)^{12},(not Lys/not Arg)^{20},(not Lys/not Arg)^{27},Arg^{40}]exendin-4; and [(not Lys/not Arg)^{20},Arg^{40}]exendin-4. Particular exendin-4 analogs include [Lys^{20},Arg^{40}]exendin-4,[His^{20},Arg^{40}]exendin-4; and [Leu^{14},Lys^{20},Arg^{40}]exendin-4.

The invention may also use truncated forms of exendin-4 or any of the exendin analogs described herein. The truncated forms may include deletions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino

acids from the N-terminus, from the C-terminus, or a combination thereof. Particular exendin-4 fragments include Exendin-4(1-31). Other fragments of exendin-4 are described in U.S. Patent Application Publication No. 2007/0037747 and have the formula:

His-Gly-Glu-Gly-Thr-X_6-Thr-Ser-Asp-Leu-Ser-Lys-Gln-X_{14}-Glu-Glu-Glu-Ala-Val-X_{20}-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-X_{30}-Pro-X_{32}

where X_6 is Phe or Tyr, X_{14} is Met, Ile or Leu, X_{20} is Lys; X_{30} is Gly or is absent; and X_{32} is Arg or is absent.

GLP-1 and GLP-1 analogs

The GLP-1 agonist used in the compositions, methods, and kits of the invention can be GLP-1 or a GLP-1 analog. In certain embodiments, the GLP-1 analog is a peptide, which can be truncated, may have one or more substitutions of the wild type sequence (e.g., the human wild type sequence), or may have other chemical modifications. GLP-1 agonists can also be non-peptide compounds, for example, as described in U.S. Patent No. 6,927,214. Particular analogs include LY548806, CJC-1131, and Liraglutide.

The GLP-1 analog can be truncated form of GLP-1. The GLP-1 peptide may be truncated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 20, or more residues from its N-terminus, its C-terminus, or a combination thereof. In certain embodiments, the truncated GLP-1 analog is the GLP-1(7-34), GLP-1(7-35), GLP-1(7-36), or GLP-1(7-37) human peptide or the C-terminal amidated forms thereof.

In other embodiments of the invention, modified forms of truncated GLP-1 peptides are used. Exemplary analogs are described in U.S. Patent No. 5,545,618 and have the amino acid sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-(Gly)-(Arg)-(Gly)

where (Gly), (Arg), and (Gly) are present or absent depending on indicated chain length, with at least one modification selected from the group consisting of (a) substitution of a neutral amino acid, Arg, or a D form of Lys for Lys at position 26 and/or 34 and/or a neutral amino acid, Lys, or a D form of Arg for Arg at position 36; (b) substitution of an oxidation-resistant amino acid for Trp at position 31; (c) substitution according to at least one of: Tyr for Val at position 16; Lys for Ser at position 18; Asp for Glu at position 21; Ser for Gly at position 22; Arg for Gln at position 23; Arg for Ala at position 24; and Gln for Lys at position 26; (d) a substitution comprising at least one of an alternative small neutral amino acid for Ala at position 8; an alternative acidic amino acid or neutral amino acid for Glu at position 9; an alternative neutral amino acid for Gly at position 10; and an alternative acidic amino acid for Asp at position 15; and (e) substitution of an alternative neutral amino acid or the Asp or N-acylated or alkylated form of His for His at position 7. With respect to modifications (a), (b), (d), and (e), the substituted amino acids may be in the D form. The amino acids substituted at position 7 can also be the N-acylated or N-alkylated amino acids. Exemplary GLP-1 analogs include [D-His^{7}]GLP-1(7-37), [Tyr^{7}]GLP-1(7-37), [N-acetyl-His^{7}]GLP-1(7-37), [N-isopropyl-His^{7}]GLP-1(7-37), [D-Ala^{8}]GLP-1(7-37), [D-Glu^{9}]GLP-1(7-37), [Asp^{9}]GLP-1(7-37), [D-Asp^{9}]GLP-1(7-37), [D-Phe^{10}]GLP-1(7-37), [Ser^{22},Arg^{23},Arg^{24},Gln^{26}]GLP-1(7-37), and [Ser^{8},Gln^{9},Tyr^{16},Lys^{18},Asp^{21}]GLP-1(7-37).

Other GLP-1 fragments are described in U.S. Patent No. 5,574,008 have the formula:

R_1-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-X-Gly-Arg-R_2

where R_1 is H_2N; H_2N-Ser; H_2N-Val-Ser; H_2N-Asp-Val-Ser; H_2N-Ser-Asp-Val-Ser; H_2N-Thr-Ser-Asp-Val-Ser; H_2N-Phe-Thr-Ser-Asp-Val-Ser; H_2N-Thr-Phe-Thr-Ser-Asp-Val-Ser; H_2N-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser; H_2N-Glu-Gly-

Thr-Phe-Thr-Ser-Asp-Val-Ser; or H_2N-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser; X is Lys or Arg; and R_2 is NH_2, OH, Gly-NH_2, or Gly-OH.

Other GLP-1 analogs, described in U.S. Patent No. 5,118,666, include the sequence His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-X, where X is Lys, Lys-Gly, or Lys-Gly-Arg.

GLP-1 analogs also include peptides of the formula: H_2N–X–CO–R_1, where R_1 is OH, OM, or $-NR_2R_3$; M is a pharmaceutically acceptable cation or a lower branched or unbranched alkyl group (e.g., C_{1-6} alkyl); R_2 and R_3 are independently selected from the group consisting of hydrogen and a lower branched or unbranched alkyl group (e.g., C_{1-6} alkyl); X is a peptide comprising the sequence His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala -Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg; NH_2 is the amine group of the amino terminus of X; and CO is the carbonyl group of the carboxy terminus of X; acid addition salts thereof; and the protected or partially protected derivatives thereof. These compounds may have insulinotropic activity exceeding that of GLP-1(1-36) or GLP-1(1-37).

Other GLP-1 analogs are described in U.S. Patent No. 5,981,488 and have the formula:

R_1-X-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Y-Gly-Gln-Ala-Ala-Lys-Z-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-R_2

where R_1 is His, D-His, desamino-His, 2-amino-His, β-hydroxy-His, homohistidine, α-fluoromethyl-His, or α-methyl-His; X is Met, Asp, Lys, Thr, Leu, Asn, Gln, Phe, Val, or Tyr; Y and Z are independently selected from Glu, Gln, Ala, Thr, Ser, and Gly; and R_2 is selected from NH_2 and Gly-OH (e.g., provided that, if R_1 is His, X is Val, Y is Glu, and Z is Glu, then R_2 is NH_2).

Other GLP-1 analogs are described in U.S. Patent No. 5,512,549 and have the formula:

R_1-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Xaa-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys(R_2)-Gly-Arg-R_3

where R_1 is 4-imidazopropionyl (des-amino-histidyl), 4-imidazoacetyl, or 4-imidazo-α, αdimethyl-acetyl; R_2, which is bound to the side chain of the Lys (e.g., through the ε amino group), is C_{6-10} unbranched acyl or is absent; R_3 is Gly-OH or NH_2; and Xaa is Lys or Arg.

Still other GLP-1 analogs are described in U.S. Patent No. 7,084,243. In one embodiment, the GLP-1 analog has the formula:

His-X_8-Glu-Gly-X_{11}-X_{12}-Thr-Ser-Asp-X_{16}-Ser-Ser-Tyr-Leu-Glu-X_{22}-X_{23}-X_{24}-Ala-X_{26}-X_{27}-Phe-Ile-Ala-X_{31}-Leu-X_{33}-X_{34}-X_{35}-X_{36}-R

where X_8 is Gly, Ala, Val, Leu, Ile, Ser, or Thr; X_{11} is Asp, Glu, Arg, Thr, Ala, Lys, or His; X_{12} is His, Trp, Phe, or Tyr; X_{16} is Leu, Ser, Thr, Trp, His, Phe, Asp, Val, Tyr, Glu, or Ala; X_{22} is Gly, Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cya; X_{23} is His, Asp, Lys, Glu, or Gln; X_{24} is Glu, His, Ala, or Lys; X_{26} is Asp, Lys, Glu, or His; X_{27} is Ala, Glu, His, Phe, Tyr, Trp, Arg, or Lys; X_{30} is Ala, Glu, Asp, Ser, or His; X_{33} is Asp, Arg, Val, Lys, Ala, Gly, or Glu; X_{34} is Glu, Lys, or Asp; X_{35} is Thr, Ser, Lys, Arg, Trp, Tyr, Phe, Asp, Gly, Pro, His, or Glu; X_{36} is Arg, Glu, or His; R is Lys, Arg, Thr, Ser, Glu, Asp, Trp, Tyr, Phe, His, $-NH_2$, Gly, Gly-Pro, or Gly-Pro-NH_2, or is deleted (e.g., provided that the polypeptide does not have the sequence of GLP-1(7-37)OH or GLP-1(7-36)-NH_2 and provided that the polypeptide is not Gly^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-36)NH_2, Val^8-GLP-1(7-37)OH, Val^8-GLP-1(7-36)NH_2, Leu^8-GLP-1(7-37)OH, Leu^8-GLP-1(7-36)NH_2, Ile^8-GLP-1(7-37)OH, Ile^8-GLP-1(7-36)NH_2, Ser^8-GLP-1(7-37)OH, Ser^8-GLP-1(7-36)NH_2, Thr^8-GLP-1(7-37)OH, or Thr^8-GLP-1(7-36)NH_2, Ala^{11}-Glp-1(7-37)OH, Ala^{11}-Glp-1(7-36)NH_2, Ala^{16}-Glp-1(7-37)OH, Ala^{16}-Glp-1(7-36)NH_2, Ala^{27}-Glp-1(7-37)OH, Ala^{27}-Glp-1(7-36)NH_2, Ala^{27}-Glp-1(7-37)OH, Ala^{27}-Glp-1(7-36)NH_2, Ala^{33}-Glp-1(7-37)OH, or Ala^{33}-Glp-1(7-36)NH_2).

In another embodiment, the polypeptide has the amino acid sequence:

His-X_8-Glu-Gly-Thr-X_{12}-Thr-Ser-Asp-X_{16}-Ser-Ser-Tyr-Leu-Glu-X_{22}-X_{23}-Ala-Ala-X_{26}-Glu-Phe-Ile-X_{30}-Trp-Leu-Val-Lys-X_{35}-Arg-R

where X_8 is Gly, Ala, Val, Leu, Ile, Ser, or Thr; X_{12} is His, Trp, Phe, or Tyr; X_{16} is Leu, Ser, Thr, Trp, His, Phe, Asp, Val, Glu, or Ala; X_{22} is Gly, Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cya; X_{23} is His, Asp, Lys, Glu, or Gln; X_{26} is Asp, Lys, Glu, or His; X_{30} is Ala, Glu, Asp, Ser, or His; X_{35} is Thr, Ser, Lys, Arg, Trp, Tyr, Phe, Asp, Gly, Pro, His, or Glu; R is Lys, Arg, Thr, Ser, Glu, Asp, Trp, Tyr, Phe, His, $-NH_2$, Gly, Gly-Pro, Gly-Pro-NH_2, or is deleted, (e.g., provided that the polypeptide does not have the sequence of GLP-1(7-37)OH or GLP-1(7-36)-NH_2 and provided that the polypeptide is not Gly^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-36)NH_2, Val^8-GLP-1(7-37)OH, Val^8-GLP-1(7-36)NH_2, Leu^8-GLP-1(7-37)OH, Leu^8-GLP-1(7-36)NH_2, Ile^8-GLP-1(7-37)OH, Ile^8-GLP-1(7-36)NH_2, Ser^8-GLP-1(7-37)OH, Ser^8-GLP-1(7-36)NH_2, Thr^8-GLP-1(7-37)OH, Thr^8-GLP-1(7-36)NH_2, Ala^{16}-GLP(7-37)OH, or Ala^{16}-GLP-1(7-36)NH_2).

In another embodiment, the polypeptide has the amino acid sequence:

His-X_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-X_{22}-X_{23}-Ala-Ala-Lys-X_{27}-Phe-Ile-X_{30}-Trp-Leu-Val-Lys-Gly-Arg-R

where X_8 is Gly, Ala, Val, Leu, Ile, Ser, or Thr; X_{22} is Gly, Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cya; X_{23} is His, Asp, Lys, Glu, or Gln; X_{27} is Ala, Glu, His, Phe, Tyr, Trp, Arg, or Lys X_{30} is Ala, Glu, Asp, Ser, or His; R is Lys, Arg, Thr, Ser, Glu, Asp, Trp, Tyr, Phe, His, $-NH_2$, Gly, Gly-Pro, or Gly-Pro-NH_2, or is deleted (e.g., provided that the polypeptide does not have the sequence of GLP-1(7-37)OH or GLP-1(7-36)NH_2 and provided that the polypeptide is not Gly^8-GLP-1(7-37)OH, Gly^8-GLP-1(7-36)NH_2, Val^8-GLP-1(7-37)OH, Val^8-GLP-1(7-36)NH_2, Leu^8-GLP-1(7-37)OH, Leu^8-GLP-1(7-36)NH_2, Ile^8-GLP-1(7-37)OH, Ile^8-GLP-1(7-36)NH_2, Ser^8-GLP-1(7-37)OH, Ser^8-GLP-1(7-36)NH_2, Thr^8-

GLP-1(7-37)OH, Thr^8-GLP-1(7-36)NH_2, Ala^{16}-GLP-1(7-37)OH, Ala^{16}-Glp-1(7-36) NH_2, Glu^{27}-Glp-1(7-37)OH, or Glu^{27}-Glp-1(7-36)NH_2.

In another embodiment, the polypeptide has the amino acid sequence:

X_7-X_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-X_{22}-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-R

where X_7 is L-His, D-His, desamino-His, 2amino-His, β-hydroxy-His, homo-His, α-fluoromethyl-His or α-methyl-His; X_8 is Gly, Ala, Val, Leu, Ile, Ser or Thr (e.g., Gly, Val, Leu, Ile, Ser, or Thr); X_{22} is Asp, Glu, Gln, Asn, Lys, Arg, Cys, or Cya, and R is $-NH_2$ or Gly(OH).

In another embodiment, the GLP-1 compound has an amino acid other than alanine at position 8 and an amino acid other than glycine at position 22. Specific examples of GLP-1 compounds include [Glu^{22}]GLP-1(7-37)OH, [Asp^{22}]GLP-1(7-37)OH, [Arg^{22}]GLP-1(7-37)OH, [Lys^{22}]GLP-1(7-37)OH, [Cya^{22}]GLP-1(7-37)OH, [Val^8,Glu^{22}]GLP-1(7-37)OH, [Val^8,Asp^{22}]GLP-1(7-37)OH, [Val^8,Arg^{22}]GLP-1(7-37)OH, [Val^8,Lys^{22}]GLP-1(7-37)OH, [Val^8,Cya^{22}]GLP-1(7-37)OH, [Gly^8,Glu^{22}]GLP-1(7-37)OH, [Gly^8,Asp^{22}]GLP-1(7-37)OH, [Gly^8,Arg^{22}]GLP-1(7-37)OH, [Gly^8,Lys^{22}]GLP-1(7-37)OH, [Gly^8,Cya^{22}]GLP-1(7-37)OH, [Glu^{22}]GLP-1(7-36)NH_2, [Asp^{22}]GLP-1(7-36)NH_2, [Arg^{22}]GLP-1(7-36)NH_2, [Lys^{22}]GLP-1(7-36)NH_2, [Cya^{22}]GLP-1(7-36)NH_2, [Val^8,Glu^{22}]GLP-1(7-36)NH_2, [Val^8,Asp^{22}]GLP-1(7-36)NH_2, [Val^8,Arg^{22}]GLP-1(7-36)NH_2, [Val^8,Lys^{22}]GLP-1(7-36)NH_2, [Val^8,Cya^{22}]GLP-1(7-36)NH_2, [Gly^8,Glu^{22}]GLP-1(7-36)NH_2, [Gly^8,Asp^{22}]GLP-1(7-36)NH_2, [Gly^8,Arg^{22}]GLP-1(7-36)NH_2, [Gly^8,Lys^{22}]GLP-1(7-36)NH_2, [Gly^8,Cya^{22}]GLP-1(7-36)NH_2, [Val^8,Lys^{23}]GLP-1(7-37)OH, [Val^8,Ala^{27}]GLP-1(7-37)OH, [Val^8,Glu^{30}]GLP-1(7-37)OH, [Gly^8,Glu^{30}]GLP-1(7-37)OH, [Val^8,His^{35}]GLP-1(7-37)OH, [Val^8,His^{37}]GLP-1(7-37)OH, [Val^8,Glu^{22},Lys^{23}]GLP-1(7-37)OH, [Val^8,Glu^{22},Glu^2]GLP-1(7-37)OH, [Val^8,Glu^{22},Ala^{27}]GLP-1(7-37)OH, [Val^8,Gly^{34},Lys^{35}]GLP-1(7-37)OH, [Val^8,His^{37}]GLP-1(7-37)OH, [Gly^8,His^{37}]GLP-1(7-37)OH.

Other GLP-1 analogs are described in U.S. Patent No. 7,101,843 and include those having the formula:

X_7-X_8-Glu-Gly-Thr-X_{12}-Thr-Ser-Asp-X_{16}-Ser-X_{18}-X_{19}-X_{20}-Glu-X_{22}-Gln-Ala-X_{25}-Lys-X_{27}-Phe-Ile-X_{30}-Trp-Leu-X_{33}-Lys-Gly-Arg-X_{37}

wherein: X_7 is L-His, D-His, desamino-His, 2-amino-His, β-hydroxy-His, homohistidine, α-fluoromethyl-His, or α-methyl-His; X_8 is Ala, Gly, Val, Leu, Ile, Ser, or Thr; X_{12} is Phe, Trp, or Tyr; X_{16} is Val, Trp, Ile, Leu, Phe, or Tyr; X_{18} is Ser, Trp, Tyr, Phe, Lys, Ile, Leu, or Val; X_{19} is Tyr, Trp, or Phe; X_{20} is Leu, Phe, Tyr, or Trp; X_{22} is Gly, Glu, Asp, or Lys; X_{25} is Ala, Val, Ile, or Leu; X_{27} is Glu, Ile, or Ala; X_{30} is Ala or Glu X_{33} is Val, or Ile; and X_{37} is Gly, His, NH_2, or is absent (e.g., provided that the compound does not have the sequence GLP-1(7-37)OH, GLP-1(7-36)-NH_2, [Gly^8]GLP-1(7-37)OH, [Gly^8]GLP-1(7-36)NH_2, [Val^8]GLP-1(7-37)OH, [Val^8]GLP-1(7-36)NH_2, [Leu^8]GLP-1(7-37)OH, [Leu^8]GLP-1(7-36)NH_2, [Ile^8]GLP-1(7-37)OH, [Ile^8]GLP-1(7-36)NH_2, [Ser^8]GLP-1(7-37)OH, [Ser^8]GLP-1(7-36)NH_2, [Thr^8]GLP-1(7-37)OH, [Thr^8]GLP-1(7-36)NH_2, [Val^8,Tyr^{12}]GLP-1(7-37)OH, [Val^8,Tyr^{12}]GLP-1(7-36)NH_2, [Val^8,Tyr^{16}]GLP-1(7-37)OH, [Val^8,Tyr^{16}]GLP-1(7-36)NH_2, [Val^8,Glu^{22}]GLP-1(7-37)OH, [Val^8,Glu^{22}]GLP-1(7-36)NH_2, [Gly^8,Glu^{22}]GLP-1(7-37)OH, [Gly^8,Glu^{22}]GLP-1(7-36)NH_2, [Val^8,Asp^{22}]GLP-1(7-37)OH, [Val^8,Asp^{22}]GLP-1(7-36)NH_2, [Gly^8,Asp^{22}]GLP-1(7-37)OH, [Gly^8,Asp^{22}]GLP-1(7-36)NH_2, [Val^8,Lys^{22}]GLP-1(7-37)OH, [Val^8,Lys^{22}]GLP-1(7-36)NH_2, [Gly^8,Lys^{22}]GLP-1(7-37)OH, [Gly^8,Lys^{22}]GLP-1(7-36)NH_2, [Leu^8,Glu^{22}]GLP-1(7-37)OH, [Leu^8,Glu^{22}]GLP-1(7-36)NH_2, [Ile^8,Glu^{22}]GLP-1(7-37)OH, [Ile^8,Glu^{22}]GLP-1(7-36)NH_2, [Leu^8,Asp^{22}]GLP-1(7-37)OH, [Leu^8,Asp^{22}]GLP-1(7-36)NH_2, [Ile^8,Asp^{22}]GLP-1(7-37)OH, [Ile^8,Asp^{22}]GLP-1(7-36)NH_2, [Leu^8,Lys^{22}]GLP-1(7-37)OH, [Leu^8,Lys^{22}]GLP-1(7-36)NH_2, [Ile^8,Lys^{22}]GLP-1(7-37)OH, [Ile^8,Lys^{22}]GLP-1(7-36)NH_2, [Ser^8,Glu^{22}]GLP-1(7-37)OH, [Ser^8,Glu^{22}]GLP-1(7-36)NH_2, [Thr^8,Glu^{22}]GLP-1(7-37)OH, [Thr^8,Glu^{22}]GLP-1(7-36)NH_2, [Ser^8,Asp^{22}]GLP-1(7-37)OH, [Ser^8,Asp^{22}]GLP-1(7-36)NH_2,

[Thr^{8},Asp^{22}]GLP-1(7-37)OH, [Thr^{8},Asp^{22}]GLP-1(7-36)NH$_2$, [Ser^{8},Lys^{22}]GLP-1(7-37)OH, [Ser^{8},Lys^{22}]GLP-1(7-36)NH$_2$, [Thr^{8},Lys^{22}]GLP-1(7-37)OH, [Thr^{8},Lys^{22}]GLP-1(7-36)NH$_2$, [Glu^{22}]GLP-1(7-37)OH, [Glu^{2}]GLP-1(7-36)NH$_2$, [Asp^{22}]GLP-1(7-37)OH, [Asp^{22}]GLP-1(7-36)NH$_2$, [Lys^{22}]GLP-1(7-37)OH, [Lys^{22}]GLP-1(7-36)NH$_2$, [Val^{8},Ala^{27}]GLP-1(7-37)OH, [Val^{8},Glu^{22},Ala^{27}]GLP-1(7-37)OH, [Val^{8},Glu^{30}]GLP-1(7-37)OH, [Val^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Gly^{8},Glu^{30}]GLP-1(7-37)OH, [Gly^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Leu^{8},Glu^{30}]GLP-1(7-37)OH, [Leu^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Ile^{8},Glu^{30}]GLP-1(7-37)OH, [Ile^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Ser^{8},Glu^{30}]GLP-1(7-37)OH, [Ser^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Thr^{8},Glu^{30}]GLP-1(7-37)OH, [Thr^{8},Glu^{30}]GLP-1(7-36)NH$_2$, [Val^{8},His^{37}]GLP-1(7-37)OH, [Val^{8},His^{37}]GLP-1(7-36)NH$_2$, [Gly^{8},His^{37}]GLP-1(7-37)OH, [Gly^{8},His^{37}]GLP-1(7-36)NH$_2$, [Leu^{8},His^{37}]GLP-1(7-37)OH, [Leu^{8},His^{37}]GLP-1(7-36)NH$_2$, [Ile^{8},His^{37}]GLP-1(7-37)OH, [Ile^{8},His^{37}]GLP-1(7-36)NH$_2$, [Ser^{8},His^{37}]GLP-1(7-37)OH, [Ser^{8},His^{37}]GLP-1(7-36)NH$_2$, [Thr^{8},His^{37}]GLP-1(7-37)OH, [Thr^{8},His^{37}]GLP-1(7-36)NH$_2$).

Other GLP-1 analogs described in U.S Patent No. 7,101,843 have the formula:

X_7-X_8-Glu-Gly-Thr-Phe-Thr-Ser-Asp-X_{16}-Ser-X_{18}-Tyr-Leu-Glu-X_{22}-Gln-Ala-X_{25}-Lys-Glu-Phe-Ile-Ala-Trp-Leu-X_{33}-Lys-Gly-Arg-X_{37}

wherein: X_7 is L-His, D-His, desamino-His, 2-amino-His, β-hydroxy-His, homohistidine, α-fluoromethyl-His, or α-methyl-His; X_8 is Gly, Ala, Val, Leu, Ile, Ser, or Thr; X_{16} is Val, Phe, Tyr, or Trp; X_{18} is Ser, Tyr, Trp, Phe, Lys, Ile, Leu, or Val; X_{22} is Gly, Glu, Asp, or Lys; X_{25} is Ala, Val, Ile, or Leu; X_{33} is Val or Ile; and X_{37} is Gly, NH$_2$, or is absent (e.g., provided that the GLP-1 compound does not have the sequence of GLP-1(7-37)OH, GLP-1(7-36)-NH$_2$, [Gly^{8}]GLP-1(7-37)OH, [Gly^{8}]GLP-1(7-36)NH$_2$, [Val^{8}]GLP-1(7-37)OH, [Val^{8}]GLP-1(7-36)NH$_2$, [Leu^{8}]GLP-1(7-37)OH, [Leu^{8}]GLP-1(7-36)NH$_2$, [Ile^{8}]GLP-1(7-37)OH, [Ile^{8}]GLP-1(7-36)NH$_2$, [Ser^{8}]GLP-1(7-37)OH, [Ser^{8}]GLP-1(7-36)NH$_2$, [Thr^{8}]GLP-1(7-37)OH, [Thr^{8}]GLP-1(7-36)NH$_2$, [Val^{8}-

Tyr^{16}]GLP-1(7-37)OH, [Val^8-Tyr^{16}]GLP-1(7-36)NH_2, [Val^8,Glu^{22}]GLP-1(7-37)OH, [Val^8,Glu^{22}]GLP-1(7-36)NH_2, [Gly^8,Glu^{22}]GLP-1(7-37)OH, [Gly^8,Glu^{22}]GLP-1(7-36)NH_2, [Val^8,Asp^{22}]GLP-1(7-37)OH, [Val^8,Asp^{22}]GLP-1(7-36)NH_2, [Gly^8,Asp^{22}]GLP-1(7-37)OH, [Gly^8,Asp^{22}]GLP-1(7-36)NH_2, [Val^8,Lys^{22}]GLP-1(7-37)OH, [Val^8,Lys^{22}]GLP-1(7-36)NH_2, [Gly^8,Lys^{22}]GLP-1(7-37)OH, [Gly^8,Lys^{22}]GLP-1(7-36)NH_2, [Leu^8,Glu^{22}]GLP-1(7-37)OH, [Leu^8,Glu^{22}]GLP-1(7-36)NH_2, [Ile^8,Glu^{22}]GLP-1(7-37)OH, [Ile^8,Glu^{22}]GLP-1(7-36)NH_2, [Leu^8,Asp^{22}]GLP1(7-37)OH, [Leu^8,Asp^{22}]GLP-1(7-36)NH_2, [Ile^8,Asp^{22}]GLP-1(7-37)OH, [Ile^8,Asp^{22}]GLP-1(7-36)NH_2, [Leu^8,Lys^{22}]GLP-1(7-37)OH, [Leu^8,Lys^{22}]GLP-1(7-36)NH_2, [Ile^8,Lys^{22}]GLP-1(7-37)OH, [Ile^8,Lys^{22}]GLP-1(7-36)NH_2, [Ser^8,Glu^{22}]GLP-1(7-37)OH, [Ser^8,Glu^{22}]GLP-1(7-36)NH_2, [Thr^8,Glu^{22}]GLP-1(7-37)OH, [Thr^8,Glu^{22}]GLP-1(7-36)NH_2, [Ser$_8$,Asp^{22}]GLP-1(7-37)OH, [Ser^8,Asp^{22}]GLP-1(7-36)NH_2, [Thr^8,Asp^{22}]GLP-1(7-37)OH, [Thr^8,Asp^{22}]GLP-1(7-36)NH_2, [Ser^8,Lys^{22}]GLP-1(7-37)OH, [Ser^8,Lys^{22}]GLP-1(7-36)NH_2, [Thr^8,Lys^{22}]GLP-1(7-37)OH, [Thr^8,Lys^{22}]GLP-1(7-36)NH_2, [Glu^{22}]GLP-1(7-37)OH, [Glu^{22}]GLP-1(7-36)NH_2, [Asp^{22}]GLP-1(7-37)OH, [Asp^{22}]GLP-1(7-36)NH_2, [Lys^{22}]GLP-1(7-37)OH, [Lys^{22}]GLP-1(7-36)NH_2).

GLP-1 analogs are also described in U.S. Patent No. 7,238,670 and have the structure:

$$A\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}X_8\text{-}X_9\text{-}Y\text{-}Z\text{-}B$$

where each of $X_{1\text{-}9}$ is a naturally or nonnaturally occurring amino acid residue; Y and Z are amino acid residues; and one of the substitutions at the α-carbon atoms of Y and Z may each independently be substituted with a primary substituent group selected from the group consisting of hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, heterocyclylalkyl, arylalkyl and heteroarylalkyl, heterocyclylalkyl said primary substituent optionally being substituted with a secondary substituent selected from a cycloalkyl, heterocyclyl, aryl, or heteroaryl group; any of said primary or secondary substituents may further be

substituted with one or more of H, alkyl, cycloalkyl, arylalkyl, aryl, heterocyclyl, heteroaryl, alkenyl, alkynyl, halo, hydroxy, mercapto, nitro, cyano, amino, acylamino, azido, guanidino, amidino, carboxyl, carboxamido, carboxamido alkyl, formyl, acyl, carboxyl alkyl, alkoxy, aryloxy, arylalkyloxy, heteroaryloxy, heterocycleoxy, acyloxy, mercapto, mercapto alkyl, mercaptoaryl, mercapto acyl, halo, cyano, nitro, azido, amino, guanidino alkyl, guanidino acyl, sulfonic, sulfonamido, alkyl sulfonyl, aryl sulfonyl or phosphonic group; wherein, the primary or secondary substitutents may optionally be bridged by covalent bonds to form one or more fused cyclic or heterocyclic systems with each other; where, the other substitution at the alpha-carbon of Y may be substituted with H, C_{1-6} alkyl, aminoalkyl, hydroxyalkyl or carboxyalkyl; where the other substitution at the alpha-carbon of Z may be substituted with hydrogen, C_{1-12} alkyl, aminoalkyl, hydroxyalkyl, or carboxyalkyl;

A and B are optionally present, where A is present and A is H, an amino acid or peptide containing from about 1-15 amino acid residues, an R group, an R–C(O) (amide) group, a carbamate group RO–C(O), a urea R_4R_5N–C(O), a sulfonamido R–SO_2, or R_4R_5N–SO_2; where R is selected from the group consisting of hydrogen, C_{1-12} alkyl, C_{3-10} cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl, and heteroaryloxyalkyl; R_4 and R_5 are each independently selected from the group consisting of H, alkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl, and heteroaryloxyalky; where the α-amino group of X_1 is substituted with H or an alkyl group, said alkyl group may optionally form a ring with A; where B is present and B is OR_1, NR_1R_2, or an amino acid or peptide containing from 1 to 15 amino acid residues (e.g., 1 to 10 or 1 to 5) terminating at the C-terminus as a carboxamide, substituted carboxamide, an ester, a free carboxylic acid, or an amino-alcohol; where R_1 and R_2 are independently chosen from H, C_{1-12} alkyl, C_{3-10} cycloalkyl, cycloalkylalkyl,

heterocyclyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl or heteroaryloxyalkyl.

Exemplary substitutions on the α-carbon atoms of Y and Z include heteroarylarylmethyl, arylheteroarylmethyl, and biphenylmethyl forming biphenylalanine residues, any of which is also optionally substituted with one or more, hydrogen, alkyl, cycloalkyl, arylalkyl, aryl, heterocyclyl, heteroaryl, alkenyl, alkynyl, halo, hydroxy, mercapto, nitro, cyano, amino, acylamino, azido, guanidino, amidino, carboxyl, carboxamido, carboxamido alkyl, formyl, acyl, carboxyl alkyl, alkoxy, aryloxy, arylalkyloxy, heteroaryloxy, heterocycleoxy, acyloxy, mercapto, mercapto alkyl, mercaptoaryl, mercapto acyl, halo, cyano, nitro, azido, amino, guanidino alkyl, guanidino acyl, sulfonic, sulfonamido, alkyl sulfonyl, aryl sulfonyl and phosphonic group.

Other embodiments include isolated polypeptides where the other substitution at the α-carbon of Y is substituted with H, methyl, or ethyl; and where the other substitution at the α-carbon of Z is substituted with H, methyl, or ethyl.

Further embodiments include isolated polypeptides as described above where X_1 is naturally or non-naturally occurring amino acid residue in which one of the substitutions at the α-carbon is a primary substituent selected from the group consisting of heterocyclylalkyl, heteroaryl, heteroarylkalkyl and arylalkyl, said primary substituent optionally being substituted with secondary substituent selected from heteroaryl or heterocyclyl; and in which the other substitution at the α-carbon is H or alkyl; X_2 is naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is an alkyl or cycloalkyl where the alkyl group may optionally form a ring with the nitrogen of X_2; and wherein the other substitution at the α-carbon is H or alkyl; X_3 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is a carboxyalkyl, bis-carboxyalkyl, sulfonylalkyl, heteroalkyl, or mercaptoalkyl; and where the other substitution at the α-carbon is hydrogen or alkyl; X_4 is a naturally or nonnaturally occurring

amino acid residue in which the α-carbon is not substituted, or in which one of the substitutions at the α-carbon is aminoalkyl, carboxyalkyl heteroarylalkyl, or heterocycylalkyl; X_5 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is an alkyl or hydroxyalkyl, and in which the other substitution at the α-carbon is hydrogen or alkyl; X_6 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is C_{1-12} alkyl, aryl, heteroaryl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, arylalkyl, or heteroarylalkyl group, and the other substitution at the α-carbon is H or alkyl; X_7 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is a hydroxylalkyl group; X_8 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at the α-carbon is C_{1-12} alkyl, hydroxylalkyl, heteroarylalkyl, or carboxamidoalkyl, and the other substitution at the α-carbon is H or alkyl; X_9 is a naturally or nonnaturally occurring amino acid residue in which one of the substitutions at α-carbon is carboxylalkyl, bis-carboxylalkyl, carboxylaryl, sulfonylalkyl, carboxylamidoalkyl, or heteroarylalkyl; and where A is H, an amino acid or peptide containing from about 1 to about 5 amino acid residues, an R group, an R–C(O) amide group, a carbamate group RO–C(O), a urea R_4R_5N–C(O), a sulfonamido R–SO_2 or a R_4R_5N–SO_2.

In certain embodiments, X_1 is His, D-His, N-Methyl-His, D-N-Methyl-His, 4-ThiazolylAla, or D-4-ThiazolylAla; X_2 is Ala, D-Ala, Pro, Gly, D-Ser, D-Asn, Nma, D-Nma, 4-ThioPro, 4-Hyp, L-2-Pip, L-2-Azt, Aib, S- or R-Iva and Acc3; X_3 is Glu, N-Methyl-Glu, Asp, D-Asp, His, Gla, Adp, Cys, or 4-ThiazolyAla; X_4 is Gly, His, Lys, or Asp; X_5 is Thr, D-Thr, Nle, Met, Nva, or L-Aoc; X_6 is Phe, Tyr, Tyr(Bzl), Tyr(3-NO_2), Nle, Trp, Phe(penta-fluoro), D-Phe(penta-fluoro), Phe(2-fluoro), Phe(3-fluoro), Phe(4-fluoro), Phe(2,3-di-fluoro), Phe(3,4-di-fluoro), Phe(3,5-di-fluoro), Phe(2,6-di-fluoro), Phe(3,4,5-tri-fluoro), Phe(2-iodo), Phe(2-OH), Phe(2-OMe), Phe(3-OMe), Phe(3-cyano), Phe(2-chloro), Phe(2-NH_2), Phe(3-NH_2), Phe(4-NH_2), Phe(4-NO_2), Phe(4-Me),

Phe(4-allyl), Phe(n-butyl), Phe(4-cyclohexyl), Phe(4-cyclohexyloxy), Phe(4-phenyloxy), 2-Nal, 2-pyridylAla, 4-thiazolylAla, 2-Thi, α-Me-Phe, D-α-Me-Phe, α-Et-Phe, D-α-Et-Phe, α-Me-Phe(2-fluoro), D-α-Me-Phe(2-fluoro), α-Me-Phe(2,3-di-fluoro), D-α-Me-Phe(2,3-di-fluoro), α-Me-Phe(2,6-di-fluoro), D-α-Me-Phe(2,6-di-fluoro), α-Me-Phe(penta-fluoro) and D-α-Me-Phe(penta-fluoro); X_7 is Thr, D-Thr, Ser, or hSer; X_8 is Ser, hSer, His, Asn, or α-Me-Ser; and X_9 is Asp, Glu, Gla, Adp, Asn, or His.

Additional embodiments include those where Y is Bip, D-Bip, L-Bip(2-Me), D-Bip(2-Me), L-Bip(2'-Me), L-Bip(2-Et), D-Bip(2-Et), L-Bip(3-Et), L-Bip(4-Et), L-Bip(2-n-propyl), L-Bip(2-n-propyl, 4-OMe), L-Bip(2-n-propyl,2'-Me), L-Bip(3-Me), L-Bip(4-Me), L-Bip(2,3-di-Me), L-Bip(2,4-di-Me), L-Bip(2,6-di-Me), L-Bip(2,4-di-Et), L-Bip(2-Me, 2'-Me), L-Bip(2-Et, 2'-Me), L-Bip(2-Et, 2'-Et), L-Bip(2-Me,4-OMe), L-Bip(2-Et,4-OMe), D-Bip(2-Et,4-OMe), L-Bip(3-OMe), L-Bip(4-OMe), L-Bip(2,4,6-tri-Me), L-Bip(2,3-di-OMe), L-Bip(2,4-di-OMe), L-Bip(2,5-di-OMe), L-Bip(3,4-di-OMe), L-Bip(2-Et,4,5-di-OMe), L-Bip(3,4-Methylene-di-oxy), L-Bip(2-Et, 4,5-Methylene-di-oxy), L-Bip(2-CH_2OH, 4-OMe), L-Bip(2-Ac), L-Bip(3-NH–Ac), L-Bip(4-NH–Ac), L-Bip(2,3-di-chloro), L-Bip(2,4-di-chloro), L-Bip(2,5-di-chloro), L-Bip(3,4-di-chloro), L-Bip(4-fluoro), L-Bip(3,4-di-fluoro), L-Bip(2,5-di-fluoro), L-Bip(3-n-propyl), L-Bip(4-n-propyl), L-Bip(2-iso-propyl), L-Bip(3-iso-propyl), L-Bip(4-iso-propyl), L-Bip(4-tert-butyl), L-Bip(3-phenyl), L-Bip(2-chloro), L-Bip(3-chloro), L-Bip(2-fluoro), L-Bip(3-fluoro), L-Bip(2-CF_3), L-Bip(3-CF_3), L-Bip(4-CF_3), L-Bip(3-NO_2), L-Bip(3-OCF_3), L-Bip(4-OCF_3), L-Bip(2-OEt), L-Bip(3-OEt), L-Bip(4-OEt), L-Bip(4-SMe), L-Bip(2-OH), L-Bip(3-OH), L-Bip(4-OH), L-Bip(2-CH_2–COOH), L-Bip(3-CH_2–COOH), L-Bip(4-CH_2–COOH), L-Bip(2-CH_2–NH_2), L-Bip(3-CH_2–NH_2), L-Bip(4-CH_2–NH_2), L-Bip(2-CH_2–OH), L-Bip(3-CH_2–OH), L-Bip(4-CH_2–OH), L-Phe[4-(1-propargyl)], L-Phe[4-(1-propenyl)], L-Phe[4-n-butyl], L-Phe[4-cyclohexyl], Phe(4-phenyloxy), L-Phe(penta-fluoro), L-2-(9,10-dihydrophenanthrenyl)-Ala, 4-(2-benzo(b)furan)-Phe, 4-(4-Dibenzofuran)-Phe, 4-(4-phenoxathiin)-Phe, 4-

(2-Benzo(b)thiophene)-Phe, , 4-(3-thiophene)-Phe, 4-(3-Quinoline)-Phe, 4-(2-naphthyl)-Phe, 4-(1-Naphthyl)-Phe, 4-(4-(3,5-dimethylisoxazole))-Phe, 4-(2,4-dimethoxypyrimidine)-Phe, homoPhe, Tyr(Bzl), Phe(3,4-di-chloro), Phe(4-Iodo), 2-Naphthyl-Ala, L-α-Me-Bip, or D-α-Me-Bip; Z is L-Bip, D-Bip, L-Bip(2-Me), D-Bip(2-Me), L-Bip(2'-Me), L-Bip(2-Et), D-Bip(2-Et), L-Bip(3-Me), L-Bip(4-Me), L-Bip(3-OMe), L-Bip(4-OMe), L-Bip(4-Et), L-Bip(2-n-propyl,2'-Me), L-Bip(2,4-di-Me), L-Bip(2-Me, 2'-Me), L-Bip(2-Me,4-OMe), L-Bip(2-Et, 4-OMe), D-Bip(2-Et,4-OMe), L-Bip(2,6-di-Me), L-Bip(2,4,6-tri-Me), L-Bip(2,3,4,5,-tetra-Me), L-Bip(3,4-di-OMe), L-Bip(2,5-di-OMe), L-Bip(3,4-Methylene-di-oxy), L-Bip(3-NH–Ac), L-Bip(2-iso-propyl), L-Bip(4-iso-propyl), L-Bip(2-Phenyl), L-Bip(4-Phenyl), L-Bip(2-fluoro), L-Bip(4-CF_3), L-Bip(4-OCF_3), L-Bip(2-OEt), L-Bip(4-OEt), L-Bip(4-SMe), L-Bip(2-CH_2–COOH), D-Bip(2-CH_2–COOH), L-Bip(2'-CH_2–COOH), L-Bip(3-CH_2–COOH), L-Bip(4-CH_2–COOH), L-Bip(2-CH_2–NH_2), L-Bip(3-CH_2–NH_2), L-Bip(4-CH_2–NH_2), L-Bip(2-CH_2–OH), L-Bip(3-CH_2–OH), L-Bip(4-CH_2–OH), L-Phe(3-Phenyl), L-Phe[4-n-Butyl], L-Phe[4-cyclohexyl], Phe(4-Phenyloxy), L-Phe(penta-fluoro), L-2-(9,10-Dihydrophenanthrenyl)-Ala, 4-(3-Pyridyl)-Phe, 4-(2-Naphthyl)-Phe, 4-(1-naphthyl)-Phe, 2-naphthyl-Ala, 2-fluorenyl-Ala, L-α-Me-Bip, D-α-Me-Bip, L-Phe(4-NO_2), or L-Phe(4-Iodo); A is H, acetyl, β-Ala, Ahx, Gly, Asp, Glu, Phe, Lys, Nva, Asn, Arg, Ser, Thr, Val, Trp, Tyr, caprolactam, Bip, Ser(Bzl), 3-pyridylAla, Phe(4-Me), Phe(penta-fluoro), 4-methylbenzyl, 4-fluorobenzyl, n-propyl, n-hexyl, cyclohexylmethyl, 6-hydroxypentyl, 2-thienylmethyl, 3-thienylmethyl, penta-fluorobenzyl, 2-naphthylmethyl, 4-biphenylmethyl, 9-anthracenylmethyl, benzyl, (S)-(2-amino-3-phenyl)propyl, methyl, 2-aminoethyl, or (S)-2-aminopropyl; and B is OH, NH_2, Trp-NH_2, 2-naphthylAla-NH_2, Phe(penta-fluoro)-NH_2, Ser(Bzl)-NH_2, Phe(4-NO_2)-NH_2, 3-pyridylAla-NH_2, Nva-NH_2, Lys-NH_2, Asp-NH_2, Ser-NH_2, His-NH_2, Tyr-NH_2, Phe-NH_2, L-Bip-NH_2, D-Ser-NH_2, Gly-OH, .beta.-Ala-OH, GABA-OH, or APA-OH.

In certain embodiments, when A is not present, and X_1 is an R group, an R–C(O) (amide) group, a carbamate group RO–C(O), a urea R_4R_5N–C(O), a sulfonamido R–SO_2, or a R_4R_5N–SO_2; wherein R is H, C_{1-12} alkyl, C_{3-10} cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl, heteroaryloxyalkyl, or heteroarylalkoxyalkyl; and where R_4 and R_5 are each independently H, C_{1-12} alkyl, C_{3-10} cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl, or heteroaryloxyalky.

In certain embodiments, when B is not present and Z is OR_1, NR_1R_2, or an amino-alcohol; where R_1 and R_2 are independently H, C_{1-12} alkyl, C_{3-10} cycloalkyl, cycloalkylalkyl, heterocycle, heterocycloalkyl, aryl, heteroaryl, arylalkyl, aryloxyalkyl, heteroarylalkyl, or heteroaryloxyalkyl. In certain embodiments, X_1 (where applicable), X_2, and X_3 are N–H or N-alkylated, (e.g., N-methylated) amino acid residues. The polypeptide may be a 10-mer to 15-mer and capable of binding to and activating the GLP-1 receptor.

Abbreviations

Nal = naphthylalanine

pGly = pentylglycine

t-BuG or = t-butylglycine

TPro = thioproline

HPro = homoproline

NmA = N-methylalanine

Cya = cysteic acid

Thi = β 2-Thienyl-Ala

hSer = homoserine

Aib = a-aminoisobutyric acid

Bip = biphenylalanine

Nle = norleucine

Ahx = 2-aminohexanoic acid

Nva = norvaline

Modified forms of GLP-1 analogs

Any of the peptide GLP-1 analogs described herein may be modified (e.g., as described herein or as known in the art. As described in U.S. Patent No. 6,924,264, the polypeptide can be bound to a polymer to increase its molecular weight. Exemplary polymers include polyethylene glycol polymers, polyamino acids, albumin, gelatin, succinyl-gelatin, (hydroxypropyl)-methacrylamide, fatty acids, polysaccharides, lipid amino acids, and dextran.

In one case, the polypeptide is modified by addition of albumin (e.g., human albumin), or an analog or fragment thereof, or the Fc portion of an immunoglobulin. Such an approach is described, for example, in U.S. Patent No. 7,271,149.

In one example, the polypeptide is modified by addition of a lipophilic substituent, as described in PCT Publication WO 98/08871. The lipophilic substituent may include a partially or completely hydrogenated cyclopentanophenathrene skeleton, a straight-chain or branched alkyl group; the acyl group of a straight-chain or branched fatty acid (e.g., a group including $CH_3(CH_2)_nCO$- or $HOOC(CH_2)_mCO$-, where n or m is 4 to 38); an acyl group of a straight-chain or branched alkane α,ω-dicarboxylic acid; $CH_3(CH_2)_p((CH_2)_q,COOH)CHNH\text{-}CO(CH_2)_2CO$-, where p and q are integers and p+q is 8 to 33; $CH_3(CH_2)_rCO\text{-}NHCH(COOH)(CH_2)_2CO$-, where r is 10 to 24; $CH_3(CH_2)_sCO\text{-}NHCH((CH_2)_2COOH)CO$-, where s is 8 to 24; $COOH(CH_2)_tCO$-, where t is 8 to 24; -$NHCH(COOH)(CH_2)_4NH\text{-}CO(CH_2)_uCH_3$, where u is 8 to 18; -$NHCH(COOH)(CH_2)_4NH\text{-}COCH((CH_2)_2COOH)NH\text{-}CO(CH_2)_wCH_3$, where w is 10 to 16; -$NHCH(COOH)(CH_2)_4NH\text{-}CO(CH_2)_2CH(COOH)NH\text{-}CO(CH_2)_xCH_3$, where x is 10 to 16; or -$NHCH(COOH)(CH_2)_4NH\text{-}CO(CH_2)_2CH(COOH)NHCO(CH_2)_yCH_3$, where y is 1 to 22.

In other embodiments, the GLP-1 peptide is modified by addition of a chemically reactive group such as a maleimide group, as described in U.S. Patent No. 6,593,295. These groups can react with available reactive functionalities on blood components to form covalent bonds and can extending the effective therapeutic in vivo half-life of the modified insulinotropic peptides. To form covalent bonds with the functional group on a protein, one can use as a chemically reactive group a wide variety of active carboxyl groups (e.g., esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS), maleimido propionic acid (MPA) maleimido hexanoic acid (MHA), and maleimido undecanoic acid (MUA).

Primary amines are the principal targets for NHS esters. Accessible α-amine groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the invention, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups.

The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (e.g., thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl is formed, which cannot be cleaved under physiological conditions.

Peptide vectors

The compounds of the invention can feature any of polypeptides described herein, for example, any of the peptides described in Table 1 (e.g., Angiopep-1 or Angiopep-2), or a fragment or analog thereof. In certain embodiments, the polypeptide may have at least 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100% identity to a polypeptide described herein. The polypeptide may have one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) substitutions relative to one of the sequences described herein. Other modifications are described in greater detail below.

The invention also features fragments of these polypeptides (e.g., a functional fragment). In certain embodiments, the fragments are capable of efficiently being transported to or accumulating in a particular cell type (e.g., liver, eye, lung, kidney, or spleen) or are efficiently transported across the BBB. Truncations of the polypeptide may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more amino acids from either the N-terminus of the polypeptide, the C-terminus of the polypeptide, or a combination thereof. Other fragments include sequences where internal portions of the polypeptide are deleted.

Additional polypeptides may be identified by using one of the assays or methods described herein. For example, a candidate polypeptide may be produced by conventional peptide synthesis, conjugated with paclitaxel and administered to a laboratory animal. A biologically-active polypeptide conjugate may be identified, for example, based on its ability to increase survival of an animal injected with tumor cells and treated with the conjugate as compared to a control which has not been treated with a conjugate (e.g., treated with the unconjugated agent). For example, a biologically active polypeptide may be identified based on its location in the parenchyma in an *in situ* cerebral perfusion assay.

Assays to determine accumulation in other tissues may be performed as well. Labelled conjugates of a polypeptide can be administered to an animal, and accumulation in different organs can be measured. For example, a

polypeptide conjugated to a detectable label (e.g., a near-IR fluorescence spectroscopy label such as Cy5.5) allows live in vivo visualization. Such a polypeptide can be administered to an animal, and the presence of the polypeptide in an organ can be detected, thus allowing determination of the rate and amount of accumulation of the polypeptide in the desired organ. In other embodiments, the polypeptide can be labelled with a radioactive isotope (e.g., ^{125}I). The polypeptide is then administered to an animal. After a period of time, the animal is sacrificed and the organs are extracted. The amount of radioisotope in each organ can then be measured using any means known in the art. By comparing the amount of a labeled candidate polypeptide in a particular organ relative to the amount of a labeled control polypeptide, the ability of the candidate polypeptide to access and accumulate in a particular tissue can be ascertained. Appropriate negative controls include any peptide or polypeptide known not to be efficiently transported into a particular cell type (e.g., a peptide related to Angiopep that does not cross the BBB, or any other peptide).

Additional sequences are described in U.S. Patent No. 5,807,980 (e.g., SEQ ID NO:102 herein), 5,780,265 (e.g., SEQ ID NO:103), 5,118,668 (e.g., SEQ ID NO:105). An exemplary nucleotide sequence encoding an aprotinin analog atgagaccag atttctgcct cgagccgccg tacactgggc cctgcaaagc tcgtatcatc cgttacttct acaatgcaaa ggcaggcctg tgtcagacct tcgtatacgg cggctgcaga gctaagcgta acaacttcaa atccgcggaa gactgcatgc gtacttgcgg tggtgcttag; SEQ ID NO:6; Genbank accession No. X04666). Other examples of aprotinin analogs may be found by performing a protein BLAST (Genbank: www.ncbi.nlm.nih.gov/BLAST/) using the synthetic aprotinin sequence (or portion thereof) disclosed in International Application No. PCT/CA2004/000011. Exemplary aprotinin analogs are also found under accession Nos. CAA37967 (GI:58005) and 1405218C (GI:3604747).

Modified polypeptides

The peptide vectors and peptide GLP-1 agonists used in the invention may have a modified amino acid sequence. In certain embodiments, the modification does not destroy significantly a desired biological activity (e.g., ability to cross the BBB or GLP-1 agonist activity). The modification may reduce (e.g., by at least 5%, 10%, 20%, 25%, 35%, 50%, 60%, 70%, 75%, 80%, 90%, or 95%), may have no effect, or may increase (e.g., by at least 5%, 10%, 25%, 50%, 100%, 200%, 500%, or 1000%) the biological activity of the original polypeptide. The modified peptide may have or may optimize a characteristic of a polypeptide, such as in vivo stability, bioavailability, toxicity, immunological activity, immunological identity, and conjugation properties.

Modifications include those by natural processes, such as posttranslational processing, or by chemical modification techniques known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side chains and the amino- or carboxy-terminus. The same type of modification may be present in the same or varying degrees at several sites in a given polypeptide, and a polypeptide may contain more than one type of modification. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made synthetically. Other modifications include pegylation, acetylation, acylation, addition of acetomidomethyl (Acm) group, ADP-ribosylation, alkylation, amidation, biotinylation, carbamoylation, carboxyethylation, esterification, covalent attachment to fiavin, covalent attachment to a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of drug, covalent attachment of a marker (e.g., fluorescent or radioactive), covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-

carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation and ubiquitination.

A modified polypeptide can also include an amino acid insertion, deletion, or substitution, either conservative or non-conservative (e.g., D-amino acids, desamino acids) in the polypeptide sequence (e.g., where such changes do not substantially alter the biological activity of the polypeptide). In particular, the addition of one or more cysteine residues to the amino or carboxy terminus of any of the polypeptides of the invention can facilitate conjugation of these polypeptides by, e.g., disulfide bonding. For example, Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), or Angiopep-7 (SEQ ID NO:112) can be modified to include a single cysteine residue at the amino-terminus (SEQ ID NOS: 71, 113, and 115, respectively) or a single cysteine residue at the carboxy-terminus (SEQ ID NOS: 72, 114, and 116, respectively). Amino acid substitutions can be conservative (i.e., wherein a residue is replaced by another of the same general type or group) or non-conservative (i.e., wherein a residue is replaced by an amino acid of another type). In addition, a non-naturally occurring amino acid can be substituted for a naturally occurring amino acid (i.e., non-naturally occurring conservative amino acid substitution or a non-naturally occurring non-conservative amino acid substitution).

Polypeptides made synthetically can include substitutions of amino acids not naturally encoded by DNA (e.g., non-naturally occurring or unnatural amino acid). Examples of non-naturally occurring amino acids include D-amino acids, an amino acid having an acetylaminomethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid, the omega amino acids of the formula $NH_2(CH_2)_nCOOH$ wherein n is 2-6, neutral nonpolar amino acids, such as sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, and norleucine. Phenylglycine may substitute for Trp, Tyr, or Phe; citrulline and

methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation conferring properties.

Analogs may be generated by substitutional mutagenesis and retain the biological activity of the original polypeptide. Examples of substitutions identified as "conservative substitutions" are shown in Table 2. If such substitutions result in a change not desired, then other type of substitutions, denominated "exemplary substitutions" in Table 3, or as further described herein in reference to amino acid classes, are introduced and the products screened.

Substantial modifications in function or immunological identity are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation. (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Histidine (His), Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe),

(2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr)

(3) acidic/negatively charged: Aspartic acid (Asp), Glutamic acid (Glu)

(4) basic: Asparaginc (Asn), Glutamine (Gln), Histidine (His), Lysine (Lys), Arginine (Arg)

(5) residues that influence chain orientation: Glycine (Gly), Proline (Pro);

(6) aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His),

(7) polar: Ser, Thr, Asn, Gln

(8) basic positively charged: Arg, Lys, His, and;

(9) charged: Asp, Glu, Arg, Lys, His

Other amino acid substitutions are listed in Table 3.

Table 2: Amino acid substitutions

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

Polypeptide derivatives and peptidomimetics

In addition to polypeptides consisting of naturally occurring amino acids, peptidomimetics or polypeptide analogs are also encompassed by the present invention and can form the peptide vectors or GLP-1 agonists used in the compounds of the invention. Polypeptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template polypeptide. The non-peptide compounds are termed "peptide mimetics" or peptidomimetics (Fauchere et al., *Infect. Immun.* 54:283-287,1986 and Evans et al., *J. Med. Chem.* 30:1229-1239, 1987). Peptide

mimetics that are structurally related to therapeutically useful peptides or polypeptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, $-CH=CH-$ (cis and trans), $-CH_2SO-$, $-CH(OH)CH_2-$, $-COCH_2-$ etc., by methods well known in the art (Spatola, *Peptide Backbone Modifications, Vega Data,* 1:267, 1983; Spatola et al., *Life Sci.* 38:1243-1249, 1986; Hudson et al., *Int. J. Pept. Res.* 14:177-185, 1979; and Weinstein, 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New York). Such polypeptide mimetics may have significant advantages over naturally occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency), reduced antigenicity, and others.

While the peptide vectors described herein may efficiently cross the BBB or target particular cell types (e.g., those described herein), their effectiveness may be reduced by the presence of proteases. Likewise, the effectiveness of GLP-1 agonists used in the invention may be similarly reduced. Serum proteases have specific substrate requirements, including L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the protease activity in serum, usually act on the first peptide bond of the polypeptide and require a free N-terminus (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). In light of this, it is often advantageous to use modified versions of polypeptides. The modified polypeptides retain the structural characteristics of the original L-amino acid polypeptides, but advantageously are not readily susceptible to cleavage by protease and/or exopeptidases.

Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., an enantiomer; D-lysine in place of L-lysine) may be used to generate more stable polypeptides. Thus, a polypeptide derivative or peptidomimetic as described herein may be all L-, all D-, or mixed D, L polypeptides. The presence of an N-terminal or C-terminal D-amino acid increases the in vivo stability of a polypeptide because peptidases cannot utilize a D-amino acid as a substrate (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Reverse-D polypeptides are polypeptides containing D-amino acids, arranged in a reverse sequence relative to a polypeptide containing L-amino acids. Thus, the C-terminal residue of an L-amino acid polypeptide becomes N-terminal for the D-amino acid polypeptide, and so forth. Reverse D-polypeptides retain the same tertiary conformation and therefore the same activity, as the L-amino acid polypeptides, but are more stable to enzymatic degradation in vitro and in vivo, and thus have greater therapeutic efficacy than the original polypeptide (Brady and Dodson, *Nature* 368:692-693, 1994 and Jameson et al., *Nature* 368:744-746, 1994). In addition to reverse-D-polypeptides, constrained polypeptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo et al., *Ann. Rev. Biochem.* 61:387-418, 1992). For example, constrained polypeptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic polypeptide. Cyclic polypeptides have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they are, of course, susceptible to endopeptidases, which do not cleave at polypeptide termini. The amino acid sequences of the polypeptides with N-terminal or C-terminal D-amino acids and of the cyclic polypeptides are usually identical to the sequences of the polypeptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

A cyclic derivative containing an intramolecular disulfide bond may be prepared by conventional solid phase synthesis while incorporating suitable S-protected cysteine or homocysteine residues at the positions selected for cyclization such as the amino and carboxy termini (Sah et al., *J. Pharm. Pharmacol.* 48:197, 1996). Following completion of the chain assembly, cyclization can be performed either (1) by selective removal of the S-protecting group with a consequent on-support oxidation of the corresponding two free SH-functions, to form a S-S bonds, followed by conventional removal of the product from the support and appropriate purification procedure or (2) by removal of the polypeptide from the support along with complete side chain de-protection, followed by oxidation of the free SH-functions in highly dilute aqueous solution.

The cyclic derivative containing an intramolecular amide bond may be prepared by conventional solid phase synthesis while incorporating suitable amino and carboxyl side chain protected amino acid derivatives, at the position selected for cyclization. The cyclic derivatives containing intramolecular -S-alkyl bonds can be prepared by conventional solid phase chemistry while incorporating an amino acid residue with a suitable amino-protected side chain, and a suitable S-protected cysteine or homocysteine residue at the position selected for cyclization.

Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a polypeptide is to add chemical groups at the polypeptide termini, such that the modified polypeptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the polypeptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of polypeptides in human serum (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an

acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular, the present invention includes modified polypeptides consisting of polypeptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

Also included by the present invention are other types of polypeptide derivatives containing additional chemical moieties not normally part of the polypeptide, provided that the derivative retains the desired functional activity of the polypeptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g., benzoyl) or a blocking group such as F-moc (fluorenylmethyl-O–CO–); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives conjugated to an antibody or other biological ligand and other types of derivatives.

Longer polypeptide sequences which result from the addition of additional amino acid residues to the polypeptides described herein are also encompassed in the present invention. Such longer polypeptide sequences can be expected to have the same biological activity and specificity (e.g., cell tropism) as the polypeptides described above. While polypeptides having a substantial number of additional amino acids are not excluded, it is recognized that some large polypeptides may assume a configuration that masks the effective sequence, thereby preventing binding to a target (e.g., a member of the LRP receptor family such as LRP or LRP2). These derivatives could act as competitive antagonists. Thus, while the present invention encompasses polypeptides or derivatives of the polypeptides described herein having an extension, desirably the extension does not destroy the cell targeting activity of the polypeptides or its derivatives.

Other derivatives included in the present invention are dual polypeptides consisting of two of the same, or two different polypeptides, as described herein, covalently linked to one another either directly or through a spacer, such as by a short stretch of alanine residues or by a putative site for proteolysis (e.g., by cathepsin, see e.g., U.S. Patent No. 5,126,249 and European Patent No. 495 049). Multimers of the polypeptides described herein consist of a polymer of molecules formed from the same or different polypeptides or derivatives thereof.

The present invention also encompasses polypeptide derivatives that are chimeric or fusion proteins containing a polypeptide described herein, or fragment thereof, linked at its amino- or carboxy-terminal end, or both, to an amino acid sequence of a different protein. Such a chimeric or fusion protein may be produced by recombinant expression of a nucleic acid encoding the protein. For example, a chimeric or fusion protein may contain at least 6 amino acids shared with one of the described polypeptides which desirably results in a chimeric or fusion protein that has an equivalent or greater functional activity.

Assays to identify peptidomimetics

As described above, non-peptidyl compounds generated to replicate the backbone geometry and pharmacophore display (peptidomimetics) of the polypeptides described herein often possess attributes of greater metabolic stability, higher potency, longer duration of action, and better bioavailability.

Peptidomimetics compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the ‘one-bead one-compound’ library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer, or small molecule libraries of compounds (Lam, *Anticancer Drug*

Des. 12:145, 1997). Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (*Proc. Natl. Acad. Sci. USA* 90:6909, 1993); Erb et al. (*Proc. Natl. Acad. Sci. USA* 91:11422, 1994); Zuckermann et al. (*J. Med. Chem.* 37:2678, 1994); Cho et al. (*Science* 261:1303, 1993); Carell et al. (*Angew. Chem, Int. Ed. Engl.* 33:2059, 1994 and *ibid* 2061); and in Gallop et al. (*Med. Chem.* 37:1233, 1994). Libraries of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13:412-421, 1992) or on beads (Lam, *Nature* 354:82-84, 1991), chips (Fodor, *Nature* 364:555-556, 1993), bacteria or spores (U.S. Patent No. 5,223,409), plasmids (Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science* 249:386-390, 1990), or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

Once a polypeptide as described herein is identified, it can be isolated and purified by any number of standard methods including, but not limited to, differential solubility (e.g., precipitation), centrifugation, chromatography (e.g., affinity, ion exchange, and size exclusion), or by any other standard techniques used for the purification of peptides, peptidomimetics, or proteins. The functional properties of an identified polypeptide of interest may be evaluated using any functional assay known in the art. Desirably, assays for evaluating downstream receptor function in intracellular signaling are used (e.g., cell proliferation).

For example, the peptidomimetics compounds of the present invention may be obtained using the following three-phase process: (1) scanning the polypeptides described herein to identify regions of secondary structure necessary for targeting the particular cell types described herein; (2) using conformationally constrained dipeptide surrogates to refine the backbone geometry and provide organic platforms corresponding to these surrogates; and (3) using the best organic platforms to display organic pharmocophores in libraries of candidates designed to mimic the desired activity of the native

polypeptide. In more detail the three phases are as follows. In phase 1, the lead candidate polypeptides are scanned and their structure abridged to identify the requirements for their activity. A series of polypeptide analogs of the original are synthesized. In phase 2, the best polypeptide analogs are investigated using the conformationally constrained dipeptide surrogates. Indolizidin-2-one, indolizidin-9-one and quinolizidinone amino acids (I^2aa, I^9aa and Qaa respectively) are used as platforms for studying backbone geometry of the best peptide candidates. These and related platforms (reviewed in Halab et al., *Biopolymers* 55:101-122, 2000 and Hanessian et al., *Tetrahedron* 53:12789-12854, 1997) may be introduced at specific regions of the polypeptide to orient the pharmacophores in different directions. Biological evaluation of these analogs identifies improved lead polypeptides that mimic the geometric requirements for activity. In phase 3, the platforms from the most active lead polypeptides are used to display organic surrogates of the pharmacophores responsible for activity of the native peptide. The pharmacophores and scaffolds are combined in a parallel synthesis format. Derivation of polypeptides and the above phases can be accomplished by other means using methods known in the art.

Structure function relationships determined from the polypeptides, polypeptide derivatives, peptidomimetics or other small molecules described herein may be used to refine and prepare analogous molecular structures having similar or better properties. Accordingly, the compounds of the present invention also include molecules that share the structure, polarity, charge characteristics and side chain properties of the polypeptides described herein.

In summary, based on the disclosure herein, those skilled in the art can develop peptides and peptidomimetics screening assays which are useful for identifying compounds for targeting an agent to particular cell types (e.g., those described herein). The assays of this invention may be developed for low-throughput, high-throughput, or ultra-high throughput screening formats. Assays of the present invention include assays amenable to automation.

Linkers

The GLP-1 agonist may be bound to the vector peptide either directly (e.g., through a covalent bond such as a peptide bond) or may be bound through a linker. Linkers include chemical linking agents (e.g., cleavable linkers) and peptides.

In some embodiments, the linker is a chemical linking agent. The GLP-1 agonist and vector peptide may be conjugated through sulfhydryl groups, amino groups (amines), and/or carbohydrates or any appropriate reactive group. Homobifunctional and heterobifunctional cross-linkers (conjugation agents) are available from many commercial sources. Regions available for cross-linking may be found on the polypeptides of the present invention. The cross-linker may comprise a flexible arm, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 carbon atoms. Exemplary cross-linkers include BS3 ([Bis(sulfosuccinimidyl)suberate]; BS3 is a homobifunctional N-hydroxysuccinimide ester that targets accessible primary amines), NHS/EDC (N-hydroxysuccinimide and N-ethyl-'(dimethylaminopropyl)carbodimide; NHS/EDC allows for the conjugation of primary amine groups with carboxyl groups), sulfo-EMCS ([N-e-Maleimidocaproic acid]hydrazide; sulfo-EMCS are heterobifunctional reactive groups (maleimide and NHS-ester) that are reactive toward sulfhydryl and amino groups), hydrazide (most proteins contain exposed carbohydrates and hydrazide is a useful reagent for linking carboxyl groups to primary amines), and SATA (N-succinimidyl-S-acetylthioacetate; SATA is reactive towards amines and adds protected sulfhydryls groups).

To form covalent bonds, one can use as a chemically reactive group a wide variety of active carboxyl groups (e.g., esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS), maleimido propionic

acid (MPA) maleimido hexanoic acid (MHA), and maleimido undecanoic acid (MUA).

Primary amines are the principal targets for NHS esters. Accessible α-amine groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the invention, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups.

The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (e.g., thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl can be formed.

In other embodiments, the linker includes at least one amino acid (e.g., a peptide of at least 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 40, or 50 amino acids). In certain embodiments, the linker is a single amino acid (e.g., any naturally occurring amino acid such as Cys). In other embodiments, a glycine-rich peptide such as a peptide having the sequence $[\text{Gly-Gly-Gly-Gly-Ser}]_n$ where n is 1, 2, 3, 4, 5 or 6 is used, as described in U.S. Patent No. 7,271,149. In other embodiments, a serine-rich peptide linker is used, as described in U.S. Patent No. 5,525,491. Serine rich peptide linkers include those of the formula $[\text{X-X-X-X-Gly}]_y$, where up to two of the X are Thr, and the remaining X are Ser, and y is 1 to 5 (e.g., Ser-Ser-Ser-Ser-Gly, where y is greater than 1). In some cases, the linker is a single amino acid (e.g., any amino acid, such as Gly or Cys).

Examples of suitable linkers are succinic acid, Lys, Glu, and Asp, or a dipeptide such as Gly-Lys. When the linker is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may, for example, form an amide bond with an amino group of the peptide or substituent. When the linker is Lys, Glu, or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may, for example, form an amide bond with a carboxyl group of the substituent. When Lys is used as the linker, a further linker may be inserted between the ε-amino group of Lys and the substituent. In one particular embodiment, the further linker is succinic acid which, e.g., forms an amide bond with the ε- amino group of Lys and with an amino group present in the substituent. In one embodiment, the further linker is Glu or Asp (e.g., which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the substituent), that is, the substituent is a N^{ε}-acylated lysine residue.

GLP-1 agonist activity assay

Determination of whether a compound has GLP-1 agonist activity can be performed using any method known in the art. Cyclic AMP (cAMP) production from cells expressing a GLP-1 receptor (e.g., a human receptor) can be measured in the presence and in the absence of a compound, where an increase in cAMP production indicates the compound to be a GLP-1 agonist.

In one example described in U.S. Patent Application Publication No. 2008/0207507, baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK-467-12A) were grown in DMEM media with the addition of 100 IU/ml penicillin, 100 μg/ml streptomycin, 5% fetal calf serum, and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was

centrifuged at 48,000×g for 15 min at 4° C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000×g for 15 min at 4° C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at −80° C.

The functional receptor assay was carried out by measuring cAMP as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 μL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM $MgCl_2$, pH 7.4) and with the following additions: 1 mM ATP, 1 μM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01% Tween-20, 0.1% BSA, 6 μg membrane preparation, 15 μg/ml acceptor beads, 20 μg/ml donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experiment. The plate was incubated in the dark with slow agitation for three hours at room temperature followed by counting in the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, Calif.).

Therapeutic applications

The compounds of the invention can be used in any therapeutic application where a GLP-1 agonist activity in the brain, or in particular tissues, is desired. GLP-1 agonist activity is associated with stimulation of insulin secretion (i.e., to act as an incretin hormone) and inhibition glucagon secretion, thereby contributing to limit postprandial glucose excursions. GLP-1 agonists can also inhibit gastrointestinal motility and secretion, thus acting as an enterogastrone and part of the "ileal brake" mechanism. GLP-1 also appears to be a physiological regulator of appetite and food intake. Because of these

actions, GLP-1 and GLP-1 receptor agonists can be used for therapy of metabolic disorders, as reviewed in, e.g., Kinzig et al., J Neurosci 23:6163-6170, 2003. Such disorders include obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, IGT, diabetic dyslipidemia, hyperlipidemia, a cardiovascular disease, and hypertension.

GLP-1 is also has neurological effects including sedative or anti-anxiolytic effects, as described in U.S. Patent No. 5,846,937. Thus, GLP-1 agonists can be used in the treatment of anxiety, aggression, psychosis, seizures, panic attacks, hysteria, or sleep disorders. GLP-1 agonists can also be used to treat Alzheimer's disease, as GLP-1 agonists have been shown to protect neurons against amyloid-β peptide and glutamate-induced apoptosis (Perry et al., Curr Alzheimer Res 2:377-85, 2005).

Other therapeutic uses for GLP-1 agonists include improving learning, enhancing neuroprotection, and alleviating a symptom of a disease or disorder of the central nervous system, e.g., through modulation of neurogenesis, and e.g., Parkinson's Disease, Alzheimer's Disease, Huntington's Disease, ALS, stroke, ADD, and neuropsychiatric syndromes (U.S. Patent No. 6,969,702 and U.S. Patent Application No. 2002/0115605). Stimulation of neurogenesis using GLP-1 agonists has been described, for example, in Bertilsson et al., J Neurosci Res 86:326-338, 2008.

Still other therapeutic uses include converting liver stem/progenitor cells into functional pancreatic cells (U.S. Patent Application Publication No. 2005/0053588); preventing beta-cell deterioration (U.S. Patent Nos. 7,259,233 and 6,569,832) and stimulation of beta-cell proliferation (U.S. Patent Application Publication No. 2003/0224983); treating obesity (U.S. Patent No. 7,211,557); suppressing appetite and inducing satiety (U.S. Patent Application Publication No. 2003/0232754); treating irritable bowel syndrome (U.S. Patent No. 6,348,447); reducing the morbidity and/or mortality associated with myocardial infarction (US Patent No. 6,747,006) and stroke (PCT Publication No. WO 00/16797); treating acute coronary syndrome characterized by an

absence of Q-wave myocardial infarction (U.S. Patent No. 7,056,887); attenuating post-surgical catabolic changes (U.S. Patent No. 6,006,753); treating hibernating myocardium or diabetic cardiomyopathy (U.S. Patent No. 6,894,024); suppressing plasma blood levels of norepinepherine (U.S. Patent No. 6,894,024); increasing urinary sodium excretion, decreasing urinary potassium concentration (U.S. Patent No. 6,703,359); treating conditions or disorders associated with toxic hypervolemia, e.g., renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, and hypertension (U.S. Patent No. 6,703,359); inducing an inotropic response and increasing cardiac contractility (U.S. Patent No. 6,703,359); treating polycystic ovary syndrome (U.S. Patent No. 7,105,489); treating respiratory distress (U.S. Patent Application Publication No. 2004/0235726); improving nutrition via a non-alimentary route, i.e., via intravenous, subcutaneous, intramuscular, peritoneal, or other injection or infusion (U.S. Patent No. 6,852,690); treating nephropathy (U.S. Patent Application Publication No. 2004/0209803); treating left ventricular systolic dysfunction, e.g., with abnormal left ventricular ejection fraction (U.S. Patent No. 7,192,922); inhibiting antro-duodenal motility, e.g., for the treatment or prevention of gastrointestinal disorders such as diarrhea, postoperative dumping syndrome and irritable bowel syndrome, and as premedication in endoscopic procedures (U.S. Patent No. 6,579,851); treating critical illness polyneuropathy (CIPN) and systemic inflammatory response syndrome (SIRS) (U.S. Patent Application Publication No. 2003/0199445); modulating triglyceride levels and treating dyslipidemia (U.S. Patent Application Publication Nos. 2003/0036504 and 2003/0143183); treating organ tissue injury caused by reperfusion of blood flow following ischemia (U.S. Patent No. 6,284,725); treating coronary heart disease risk factor (CHDRF) syndrome (U.S. Patent No. 6,528,520); and others.

Administration and dosage

The present invention also features pharmaceutical compositions that contain a therapeutically effective amount of a compound of the invention. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (*Science* 249:1527-1533, 1990).

The pharmaceutical compositions are intended for parenteral, intranasal, topical, oral, or local administration, such as by a transdermal means, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered parenterally (e.g., by intravenous, intramuscular, or subcutaneous injection), or by oral ingestion, or by topical application or intraarticular injection at areas affected by the vascular or cancer condition. Additional routes of administration include intravascular, intra-arterial, intratumor, intraperitoneal, intraventricular, intraepidural, as well as nasal, ophthalmic, intrascleral, intraorbital, rectal, topical, or aerosol inhalation administration. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants or components. Thus, the invention provides compositions for parenteral administration that comprise the above mention agents dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. The invention also provides compositions for oral delivery, which may contain inert ingredients such as binders or fillers for the formulation of a tablet, a capsule, and the like.

Furthermore, this invention provides compositions for local administration, which may contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, and the like.

These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

The compositions containing an effective amount can be administered for prophylactic or therapeutic treatments. In prophylactic applications, compositions can be administered to a subject with a clinically determined predisposition or increased susceptibility to a metabolic disorder or neurological disease. Compositions of the invention can be administered to the patient (e.g., a human) in an amount sufficient to delay, reduce, or preferably prevent the onset of clinical disease. In therapeutic applications, compositions are administered to a subject (e.g., a human) already suffering from disease (e.g., a metabolic disorder such as those described herein, or a neurological disease) in an amount sufficient to cure or at least partially arrest the symptoms of the condition and its complications. An amount adequate to accomplish this purpose is defined as a "therapeutically effective amount," an amount of a compound sufficient to substantially improve some symptom associated with a disease or a medical condition. For example, in the treatment of a metabolic disorder (e.g., those described herein), an agent or compound which decreases, prevents, delays, suppresses, or arrests any symptom of the disease or condition

would be therapeutically effective. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is less severe or recovery is accelerated in an individual.

Exendin-4 is typically taken twice daily at either 5 μg or 10 μg per dose for treatment of diabetes. The compounds of the invention may be administered in equivalent doses of as specified for exendin-4, may be administered in higher equivalent doses (e.g., 10%, 25%, 50%, 100%, 200%, 500%, 1000% greater doses), or can be administered in lower equivalent doses (e.g., 90%, 75%, 50%, 40%, 30%, 20%, 15%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the equivalent dose). Amounts effective for this use may depend on the severity of the disease or condition and the weight and general state of the patient, but generally range from about 0.05 μg to about 1000 μg (e.g., 0.5-100 μg) of an equivalent amount of exendin-4 the agent or agents per dose per patient. Suitable regimes for initial administration and booster administrations are typified by an initial administration followed by repeated doses at one or more hourly, daily, weekly, or monthly intervals by a subsequent administration. The total effective amount of an agent present in the compositions of the invention can be administered to a mammal as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which multiple doses are administered over a more prolonged period of time (e.g., a dose every 4-6, 8-12, 14-16, or 18-24 hours, or every 2-4 days, 1-2 weeks, once a month). Alternatively, continuous intravenous infusion sufficient to maintain therapeutically effective concentrations in the blood are contemplated.

The therapeutically effective amount of one or more agents present within the compositions of the invention and used in the methods of this invention applied to mammals (e.g., humans) can be determined by the

ordinarily-skilled artisan with consideration of individual differences in age, weight, and the condition of the mammal. Because certain compounds of the invention exhibit an enhanced ability to cross the BBB, the dosage of the compounds of the invention can be lower than (e.g., less than or equal to about 90%, 75%, 50%, 40%, 30%, 20%, 15%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of) the equivalent dose of required for a therapeutic effect of the unconjugated GLP-1 agonist. The agents of the invention are administered to a subject (e.g. a mammal, such as a human) in an effective amount, which is an amount that produces a desirable result in a treated subject (e.g. reduction in glycemia, reduced weight gain, increased weight loss, and reduced food intake). Therapeutically effective amounts can also be determined empirically by those of skill in the art.

The patient may also receive an agent in the range of about 0.05 to 1,000 μg equivalent dose as compared to exendin-4 per dose one or more times per week (e.g., 2, 3, 4, 5, 6, or 7 or more times per week), 0.1 to 2,500 (e.g., 2,000, 1,500, 1,000, 500, 100, 10, 1, 0.5, or 0.1) μg dose per week. A patient may also receive an agent of the composition in the range of 0.1 to 3,000 μg per dose once every two or three weeks.

Single or multiple administrations of the compositions of the invention comprising an effective amount can be carried out with dose levels and pattern being selected by the treating physician. The dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in the patient, which may be monitored throughout the course of treatment according to the methods commonly practiced by clinicians or those described herein.

The compounds of the present invention may be used in combination with either conventional methods of treatment or therapy or may be used separately from conventional methods of treatment or therapy.

When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or

concurrently to an individual. Alternatively, pharmaceutical compositions according to the present invention may be comprised of a combination of a compound of the present invention in association with a pharmaceutically acceptable excipient, as described herein, and another therapeutic or prophylactic agent known in the art.

Example 1

Synthesizing GLP-1 agonist-Angiopep conjugates

The exemplary GLP-1 conjugates, exendin-4-cysAn2 N-terminal, and Exendin-4-cysAn2 C-terminal, and Angiopep-1/Exendin 4 conjugates were made by conjugating [Lys(maleimido hexanoic acid)^{39}]exendin-4 to the sulfide in cys-An2 (SEQ ID NO:113), in An2-cys (SEQ ID NO:114), or in Angiopep-1 (SEQ ID NO:67) in 1x PBS buffer for 1 hour. This resulted in production of exendin-4/Angiopep conjugates, as shown in Figure 2.

A second set of exendin-4/Angiopep conjugates was made by reacting Angiopep-2 having maleimido propionic acid (MPA), maleimido hexanoic acid (MHA), or maleimido undecanoic acid (MUA) bound to its N-terminus with [Cys^{32}]Exendin-4 to form a conjugate, as shown in Figure 3.

Example 2

Brain uptake of exendin-4/Angiopep-2 conjugates in situ

To measure brain uptake of the exendin-4/Angiopep-2 conjugates, we used an in situ perfusion assay. The assay, which is described in U.S. Patent Application Publication No. 2006/0189515, is performed as follows. The uptake of labeled exendin-4 and the exendin-4/Angiopep-2 conjugates was measured using the in situ brain perfusion method adapted in our laboratory for the study of drug uptake in the mouse brain (Dagenais et al., J Cereb Blood Flow Metab. 20:381-6, 2000; Cisternino et al., Pharm Res **18,** 183-190, 2001). Briefly, the right common carotid artery of mice anesthetized with ketamine/xylazine (140/8 mg/kg i.p.) was exposed and ligated at the level of the

bifurcation of the common carotid, rostral to the occipital artery. The common carotid was then catheterized rostrally with polyethylene tubing filled with heparin (25 U/ml) and mounted on a 26-gauge needle. The syringe containing the perfusion fluid ([^{125}I]-proteins or [^{125}I]-peptides in Krebs/bicarbonate buffer at pH 7.4, gassed with 95% O_2 and 5% CO_2) was placed in an infusion pump (Harvard pump PHD 2000; Harvard Apparatus) and connected to the catheter. Prior to the perfusion, the contralateral blood flow contribution was eliminated by severing the heart ventricles. The brain was perfused for 5 min at a flow rate of 1.15 ml/min. After perfusion of radiolabeled molecules, the brain was further perfused for 60 s with Krebs buffer, to wash away excess [^{125}I]-proteins. Mice were then decapitated to terminate perfusion and the right hemisphere was isolated on ice before being subjected to capillary depletion. Aliquots of homogenates, supernatants, pellets, and perfusates were taken to measure their contents and to evaluate the apparent volume of distribution.

From these experiments, brain distribution of both exendin-4/Angiopep-2 conjugates was increased 15-50 fold over that of unconjugated exendin-4. The brain distribution of exendin-4 was observed at 0.2 ml/100 g/2 min, whereas the conjugate modified at its N-terminal was observed at 3 ml/100 g/2 min, and the conjugate modified at its C-terminal was observed at 10 ml/100 g/2 min. Results are shown in Figure 4.

Example 3

Treatment of obese mice with exendin-4/Angiopep-2 conjugates

Obese mice (ob/ob mice) were administered the [Lys^{39}-MHA]exendin-4/Angiopep-2-Cys-NH_2 conjugate (Exen-An2).

In vivo study to determine the efficacy of Exendin-4-Angiopep-2 conjugate

Groups	Dose (µg/kg)	Dose (nmol/kg)	Dose (µg/mouse)	mice/group	Q1Dx 28 days (Total amount µg)
Control	0	0	0	5	0
Exendin-4					
	3	0.72	0.18	5	20.16
	30	7.2	1.8	5	201.6
Exen-An2					
	4.8	0.72	0.288	5	32.256
	48	7.2	2.88	5	322.56

A 1.6 µg/kg dose of Exen-An2 is equivalent to a 1 µg/kg dose of exendin-4. The body weight of each mouse was measured daily. Food intake was estimated based on the mean values for each group, and glycemia was measured one hour following treatment. After 10 days of treatment, body weight gain and food intake of mice treated at the higher doses of either exendin-4 or the conjugate are lower than the control (Figure 5). Food intake was also reduced in the mice receiving the higher doses of either exendin-4 or the conjugate (Figure 6) as compared to the control.

Glycemia measurements showed that the lower dose of the conjugate had the same effect as the higher doses of either exendin-4 or Exen-An2 (Figure 7). Thus, a similar effect of 1/10 the dosage on glycemia is observed using the conjugate, as compared to exendin-4.

Example 4

Generation of an Exendin-4-Angioep-2 dimer conjugate

Using the conjugation chemistry described herein or similar chemistry, an Exendin-4-Angiopep-2 dimer was generated having the structure shown in Figure 8A. Briefly, the amine group in the C-terminal lysine of $[Lys^{39}]$Exendin-4 was conjugated to an Angiopep-2 dimer through an MHA linker at the N-terminal threonine of the first Angiopep-2 peptide. A N-Succinimidyl-S-acetylthiopropionate (SATP) linker was attached to an Angiopep-2-Cys peptide at its N-terminus. Through this cysteine, the

Angiopep-2-Cys peptide was conjugated to a second Angiopep-2 peptide, which had been modified to contain an MPA linker. The dimer was the linked to the [Lys^{39}]Exendin-4 through an MHA linker A control molecule (Exen-S4) was also generated using a scrambed form of Angiopep-2 conjugated at its N-terminal to the cysteine of [Cys^{32}]Exendin-4 through an MHA linker (Figure 8B). These conjugates were prepared as trifluoroacetate (TFA) salts.

Example 5

Characterization of an exendin-4-Angiopep-2 dimer conjugate

Brain uptake of the exemplary GLP-1 agonist, exendin-4, was measured in situ when unconjugated, conjugated to a single Angiopep-2, conjugated to a scrambled Angiopep-2 (S4), or conjugated to a dimeric form of Angiopep-2. The experiments were performed as described in Example 2 above.

From these results, we observed that conjugation of the exendin-4 analog to the dimeric form of Angiopep-2 results in a conjugate with a surprisingly greater ability to cross the BBB as compared to either the unconjugated exendin-4 or to the exendin-4 conjugated to a single Angiopep-2 (Figure 9).

We also tested the ability of the exendin-4-Angiopep-2 dimer conjugate to reduce glycemia in DIO mice. Mice were injected with a bolus containing a control, exendin-4, or the exendin-4-Angiopep-2 dimer conjugate. Mice receiving either exendin-4 or the conjugate exhibited reduced glycemia as compared to mice receiving the control (Figure 10).

Example 6

Characterization of an exendin-4-Angiopep-2 dimer conjugate

Brain uptake of the exemplary GLP-1 agonist, exendin-4, was measured in situ when unconjugated, conjugated to a single Angiopep-2, conjugated to S4, or conjugated to a dimeric form of Angiopep-2. The experiments were performed as described in Example 2 above.

From these results, we observed that conjugation of the exendin-4 analog to the dimeric form of Angiopep-2 results in a conjugate with a surprisingly greater ability to cross the BBB as compared to either the unconjugated exendin-4 or to the exendin-4 conjugated to a single Angiopep-2 (Figure 8).

We also tested the ability of the exendin-4-Angiopep-2 dimer conjugate to reduce glycemia in DIO mice. Mice were injected with a bolus containing a control, exendin-4, or the exendin-4-Angiopep-2 dimer conjugate. Mice receiving either exendin-4 or the conjugate exhibited reduced glycemia as compared to mice receiving the control (Figure 9).

Other embodiments

All patents, patent applications, including U.S. Provisional Application No. 61/105,618, filed October 15, 2008, and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent, patent application, or publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

CLAIMS

1. A compound having the formula

A-X-B

where A is a peptide capable of crossing the blood-brain barrier; X is a linker; and B is a GLP-1 agonist, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein A comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NOS:1-105, 107-111, 113, and 114.

3. The compound of claim 2, wherein A is a polypeptide has an amino acid sequence at least 70% identical to a sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), cys-Angiopep-2 (SEQ ID NO:113), and Angiopep-2-cys (SEQ ID NO:114).

4. The compound of claim 3, wherein said sequence identity is at least 90%.

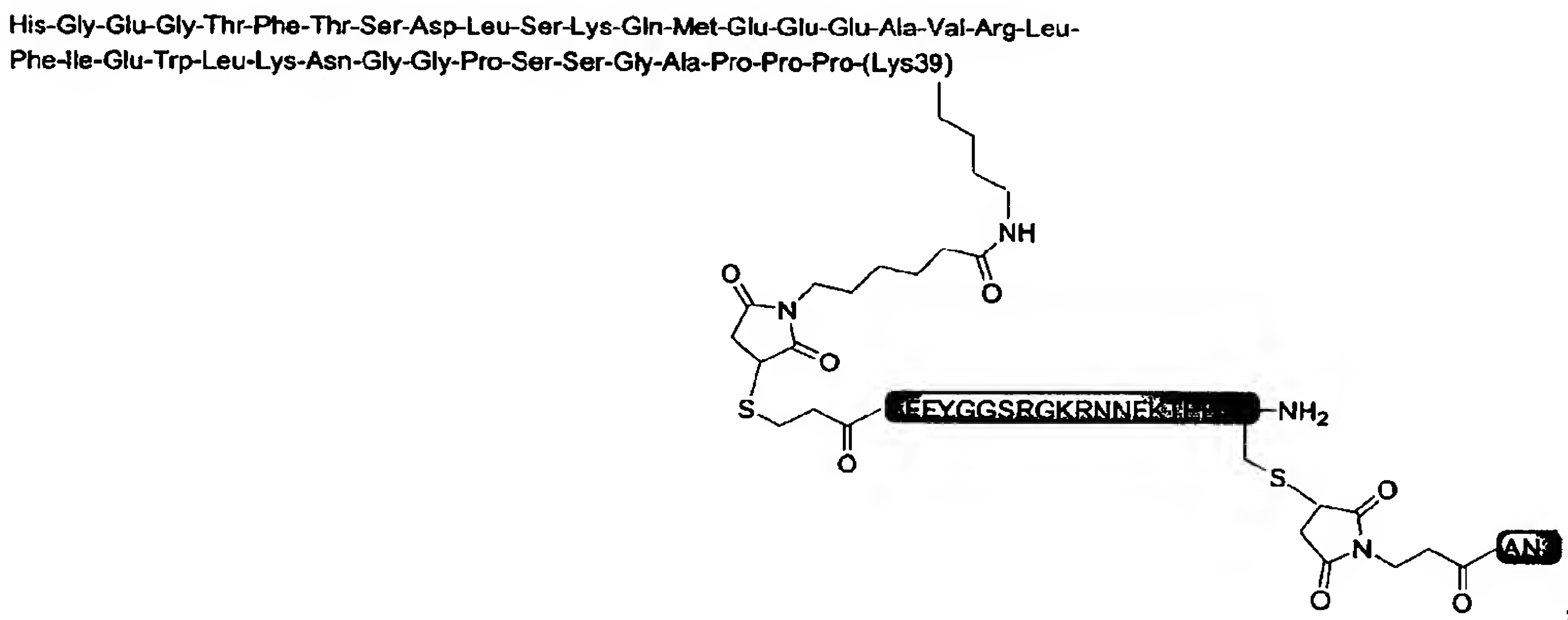
5. The compound of claim 4, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), cys-Angiopep-2 (SEQ ID NO:113), and Angiopep-2-cys (SEQ ID NO:114).

6. The compound of claim 5, wherein said polypeptide consists of an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), cys-Angiopep-2 (SEQ ID NO:113), and Angiopep-2-cys (SEQ ID NO:114).

7. The compound of any of claims 1-6, wherein A is a dimeric polypeptide.

8. The compound of claim 7, wherein A is a dimer of Angiopep-2.

9. The compound of claim 8, wherein said compound comprises the structure:



,

or a pharmaceutically acceptable salt thereof

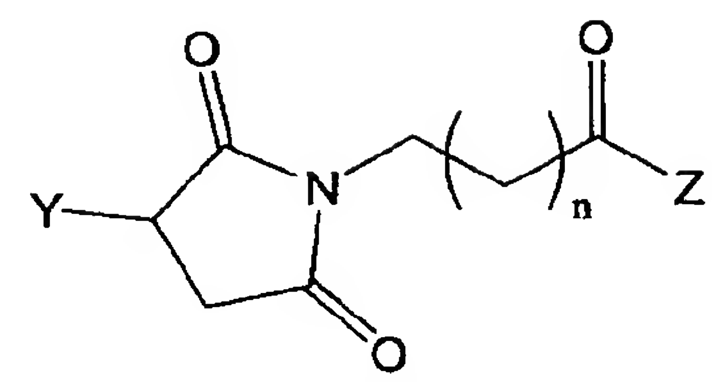
10. The compound of claim 1, wherein B comprises a polypeptide.

11. The compound of claim 10, wherein B comprises exendin-4, or an analog or fragment thereof having GLP-1 agonist activity.

12. The compound of claim 10, wherein B is exendin-4, [Lys^{39}]exendin-4, or [Cys^{32}]exendin-4.

13. The compound of claim 12, wherein A comprises Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), cys-Angiopep-2 (SEQ ID NO:113), or Angiopep-2-cys (SEQ ID NO:114).

14. The compound of any of claims 1-13, wherein X has the formula:



where n is an integer between 2 and 15; and either Y is a thiol on A and Z is a primary amine on B or Y is a thiol on B and Z is a primary amine on A.

15. The compound of claim 14, wherein n is 3, 6, or 11.

16. The compound of claim 15, wherein A is cys-AngioPep-2 (SEQ ID NO:113), AngioPep-2-cys-NH_2 (SEQ of ID NO:114) and B is [Lys^{39}]exendin-4, and Y is the thiol group on the cysteine of A, and Z is the ε-amine of Lys^{39} of B.

17. The compound of claim 1, wherein B is polypeptide and X is peptide bond.

18. The compound of claim 1, wherein B is a polypeptide, X is at least one amino acid, and A and B are each covalently bonded to X by a peptide bond.

19. A nucleic acid molecule encoding the compound of claim 17 or 18.

20. A vector comprising the nucleic acid molecule of claim 19, wherein said nucleic acid is operably linked to a promoter.

21. A method of making a compound of claim 17 or 18, said method comprising expressing a polypeptide encoded by the vector of claim 20 in a cell, and purifying said polypeptide.

22. A method of making a compound of claim 17 or 18, said method comprising synthesizing said compound on solid support.

23. A method of treating a subject having a metabolic disorder, said method comprising administering a compound of any of claims 1-18 in an amount sufficient to treat said disorder.

24. The method of claim 23, wherein said amount sufficient is less than 50% of the amount required for an equivalent dose of the GLP-1 agonist when not conjugated to the peptide vector.

25. The method of claim 24, wherein said amount is less than 15%.

26. The method of claim 23, wherein said metabolic disorder is diabetes, obesity, diabetes as a consequence of obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, impaired glucose tolerance (IGT), diabetic dyslipidemia, hyperlipidemia, a cardiovascular disease, or hypertension.

27. The method of claim 23, wherein said disorder is diabetes.

28. The method of claim 27, wherein said disorder is type II diabetes.

29. The method of claim 23 wherein said disorder obesity.

30. A method of reducing food intake by, or reducing body weight of, a subject, said method comprising administering a compound of any of claims 1-18 to a subject in an amount sufficient to reduce food intake or reduce body weight.

31. The method of claim 30, wherein said subject is overweight or obese.

32. The method of claim 30, wherein said subject is bulimic.

33. A method of treating or preventing a disorder selected from the group consisting of anxiety, movement disorder, aggression, psychosis, seizures, panic attacks, hysteria, sleep disorders, Alzheimer's disease, and Parkinson's disease, said method comprising administering a compound of any of claims 1-18 to a subject in an amount sufficient to treat or prevent said disorder.

34. A method of increasing neurogenesis in a subject, said method comprising administering to said subject and effective amount of a compound of any of claims 1-18 to said subject.

35. The method of claim 34, wherein said subject is suffering from Parkinson's Disease, Alzheimer's Disease, Huntington's Disease, ALS, stroke, ADD, or a neuropsychiatric syndrome.

36. The method of claim 34, wherein said increase in neurogenesis improves learning or enhances neuroprotection in said subject.

37. A method for converting liver stem/progenitor cells into functional pancreatic cells; preventing beta-cell deterioration and stimulation of beta-cell proliferation; treating obesity; suppressing appetite and inducing satiety; treating irritable bowel syndrome; reducing the morbidity and/or mortality associated with myocardial infarction and stroke; treating acute coronary syndrome characterized by an absence of Q-wave myocardial infarction;

attenuating post-surgical catabolic changes; treating hibernating myocardium or diabetic cardiomyopathy; suppressing plasma blood levels of norepinepherine; increasing urinary sodium excretion, decreasing urinary potassium concentration; treating conditions or disorders associated with toxic hypervolemia, e.g., renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, and hypertension; inducing an inotropic response and increasing cardiac contractility; treating polycystic ovary syndrome; treating respiratory distress; improving nutrition via a non-alimentary route, i.e., via intravenous, subcutaneous, intramuscular, peritoneal, or other injection or infusion; treating nephropathy; treating left ventricular systolic dysfunction (e.g., with abnormal left ventricular ejection fraction); inhibiting antro-duodenal motility (e.g., for the treatment or prevention of gastrointestinal disorders such as diarrhea, postoperative dumping syndrome and irritable bowel syndrome, and as premedication in endoscopic procedures ; treating critical illness polyneuropathy (CIPN) and systemic inflammatory response syndrome (SIRS; modulating triglyceride levels and treating dyslipidemia; treating organ tissue injury caused by reperfusion of blood flow following ischemia; or treating coronary heart disease risk factor (CHDRF) syndrome in a subject, said method comprising administering and effective amount of a compound of any of claims 1-18 to said subject.

38. A method of increasing GLP-1 receptor activity in a subject, said method comprising administering a compound of any of claims 1-18 to a subject in an amount sufficient to increase GLP-1 receptor activity.

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Peptides	Amino acid sequence
Exendin-4 native	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS
Exendin-4-Lys(MHA)	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPP**K-(MHA)**
(Cys32)-Exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGP**C**SGAPPPS

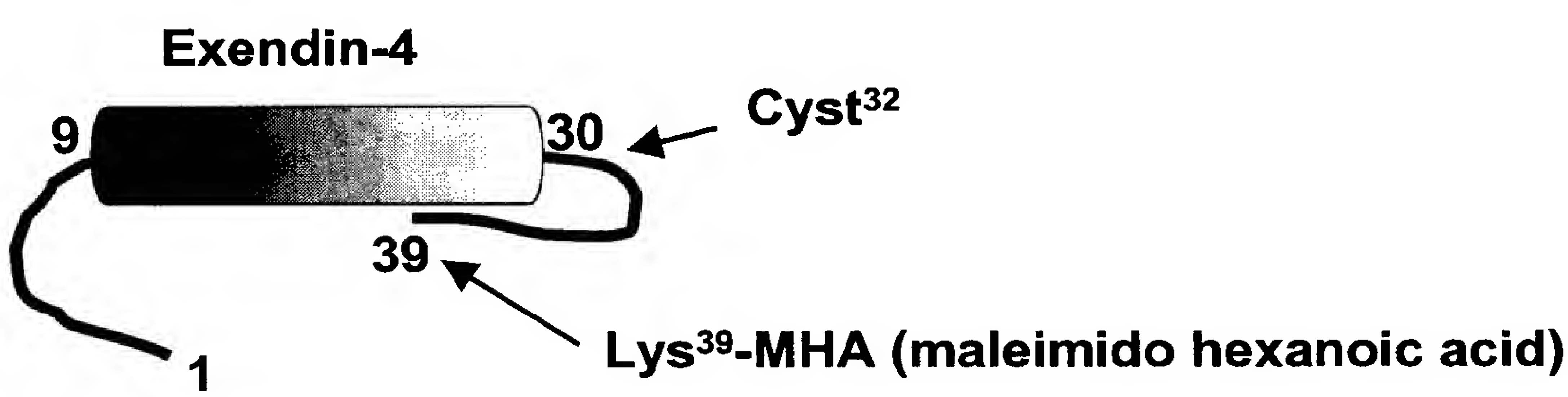
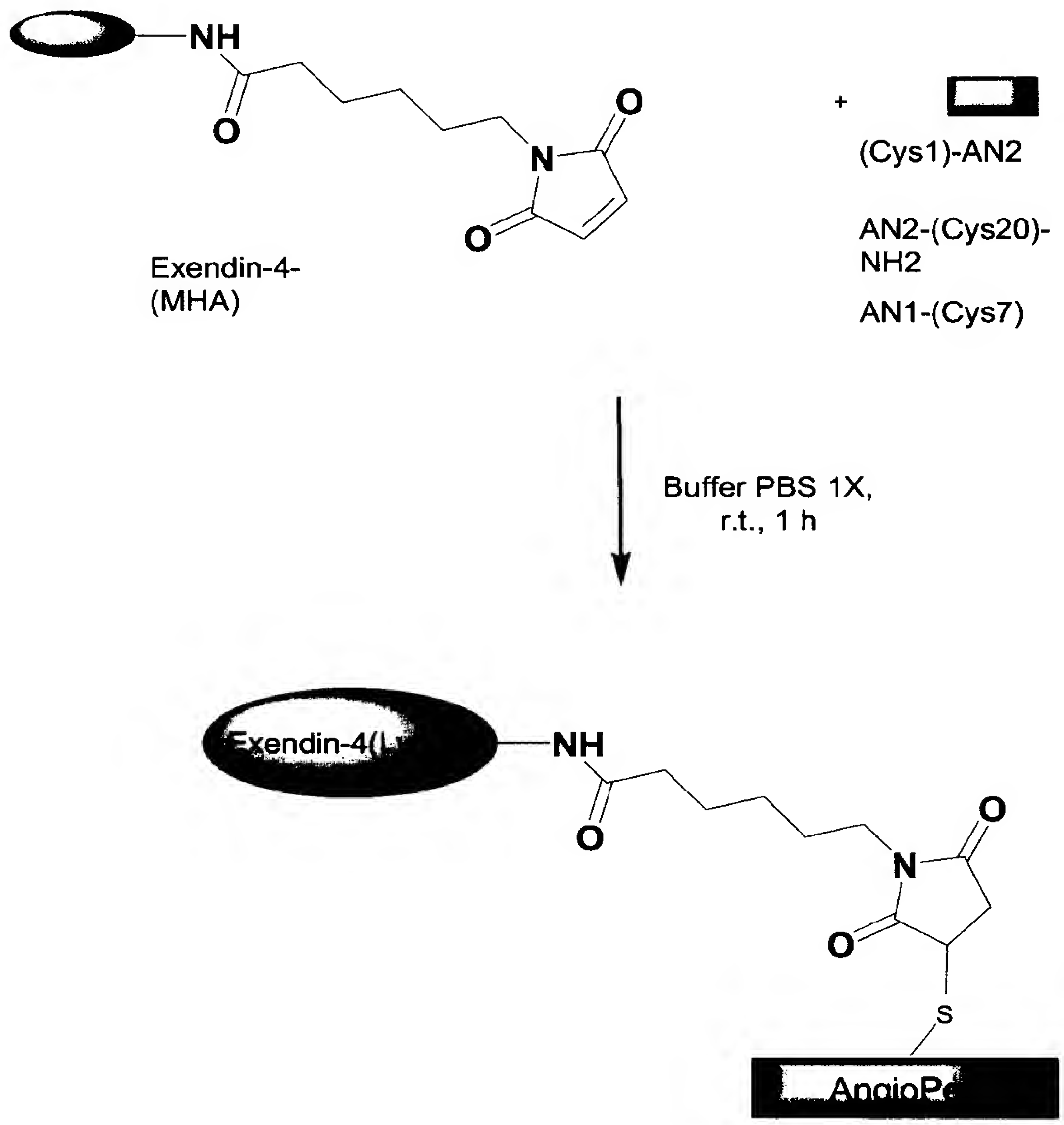


Figure 1

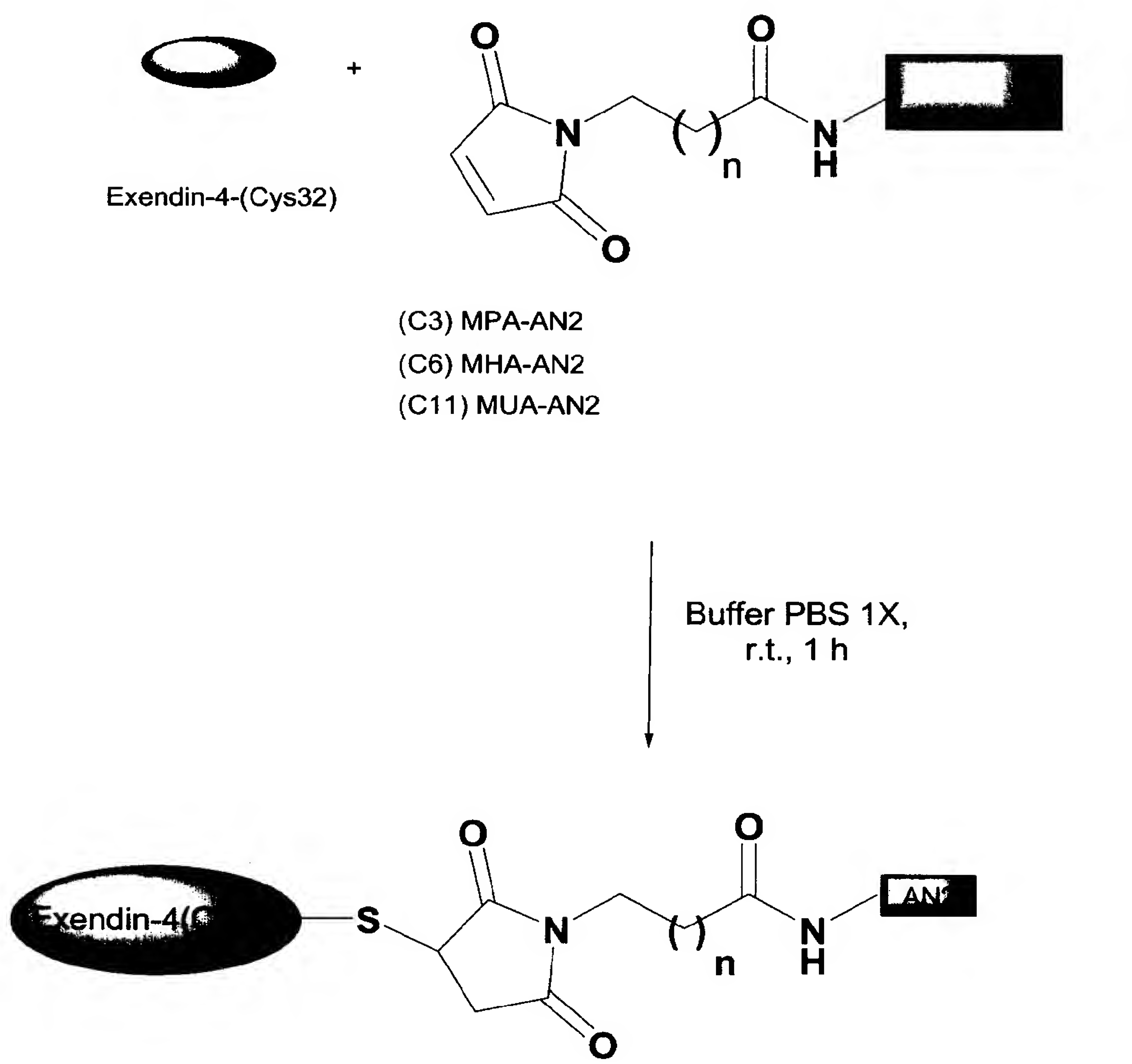
PATENT
ATTORNEY DOCKET NO. V82690WO



Series I	Qtty (mg)	Purity (%)	MW (g/mol)
Ex-4-AN2 (N-Terminal)	4.9	> 95	6825.45
Ex-4-AN2 (C-Terminal)	5.5	> 95	6825.48
Ex-4-AN1	5.3	> 85	6739.38

Figure 2

PATENT
ATTORNEY DOCKET NO. V82690WO



(C3) MPA-AN2
(C6) MHA-AN2
(C11) MUA-AN2

(C3)
(C6)
(C11)

Series II	Qtty (mg)	Purity (%)	MW (g/mol)
Ex-4-(C3)-AN2	13.5	> 98	6656.21
Ex-4-(C6)-AN2	4.8	> 98	6768.43
Ex-4-(C11)-AN2	8.8	> 90	6698.17

Figure 3

PATENT
ATTORNEY DOCKET NO. V82690WO

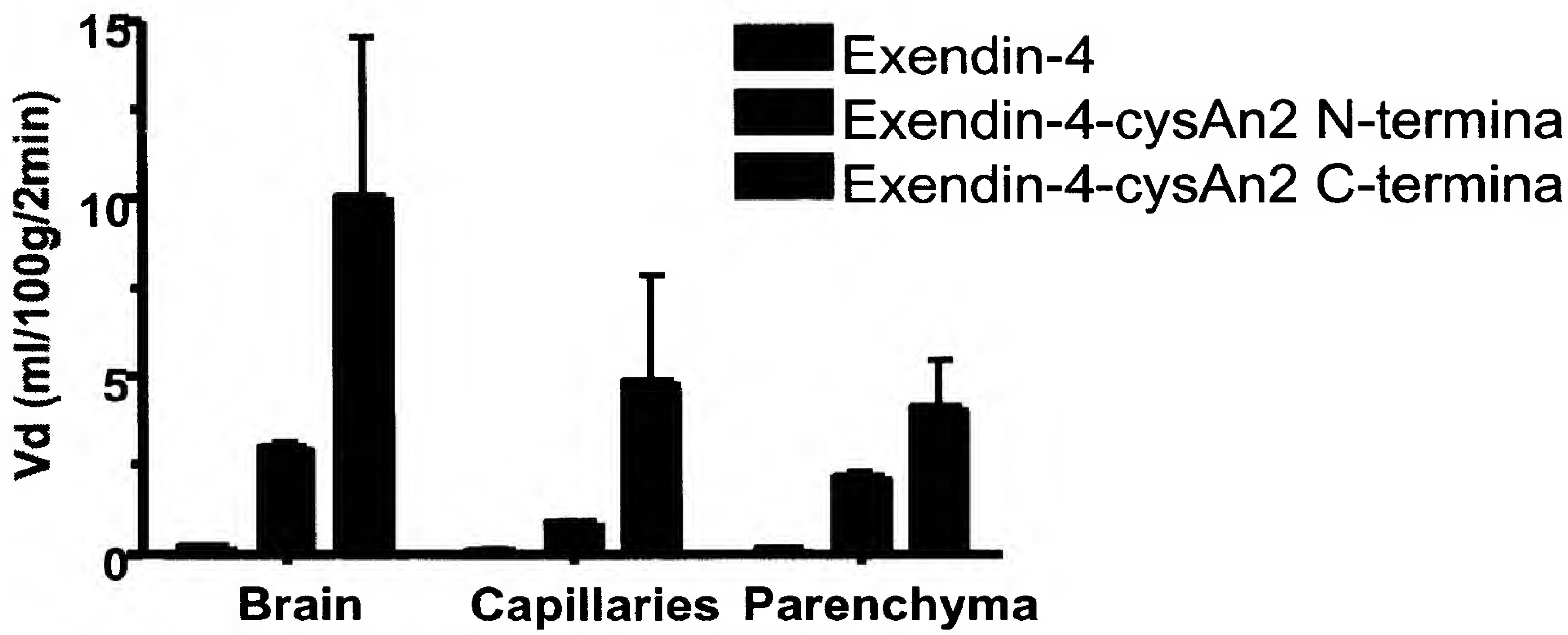
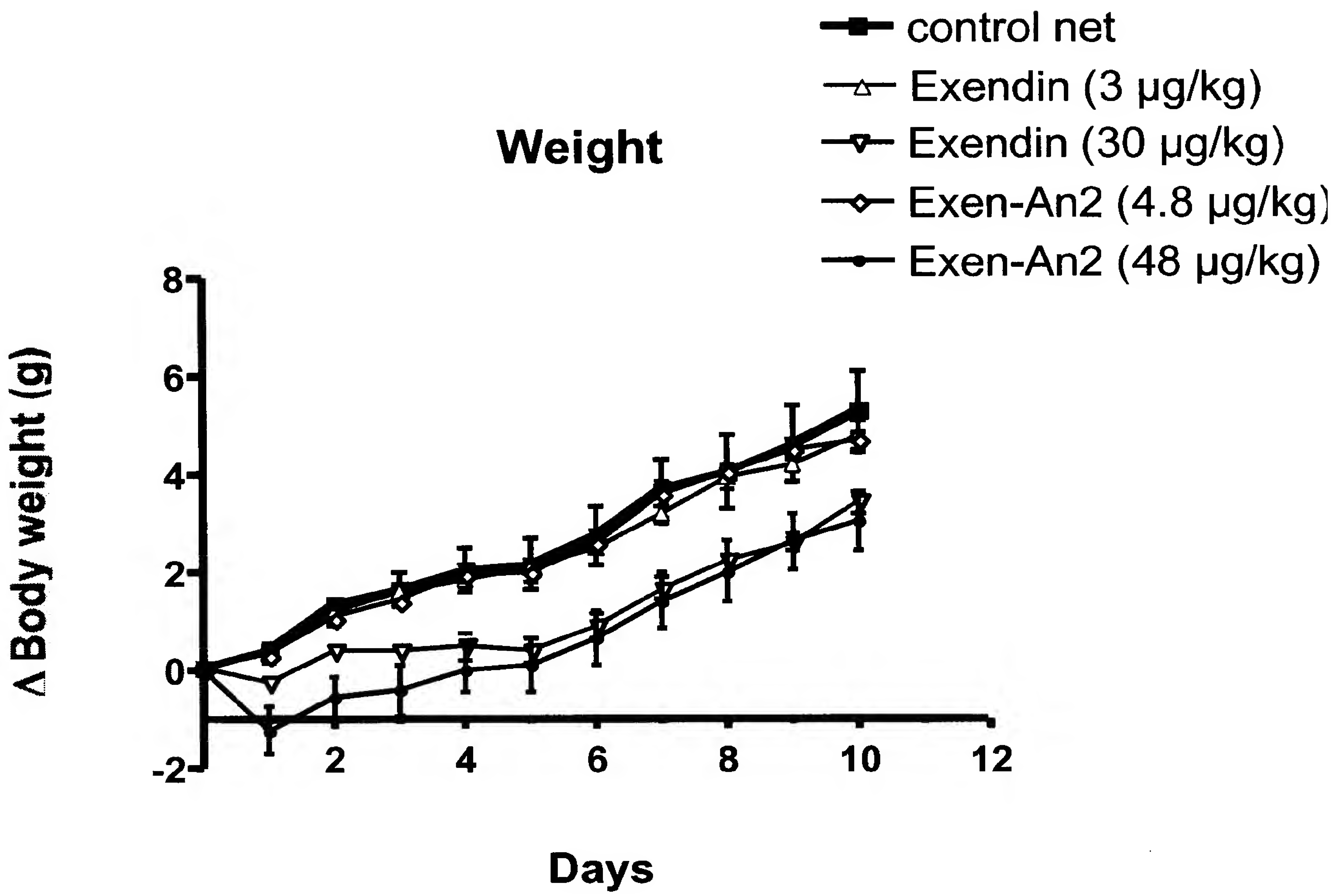


Figure 4

PATENT
ATTORNEY DOCKET NO. V82690WO



First treatment

Figure 5

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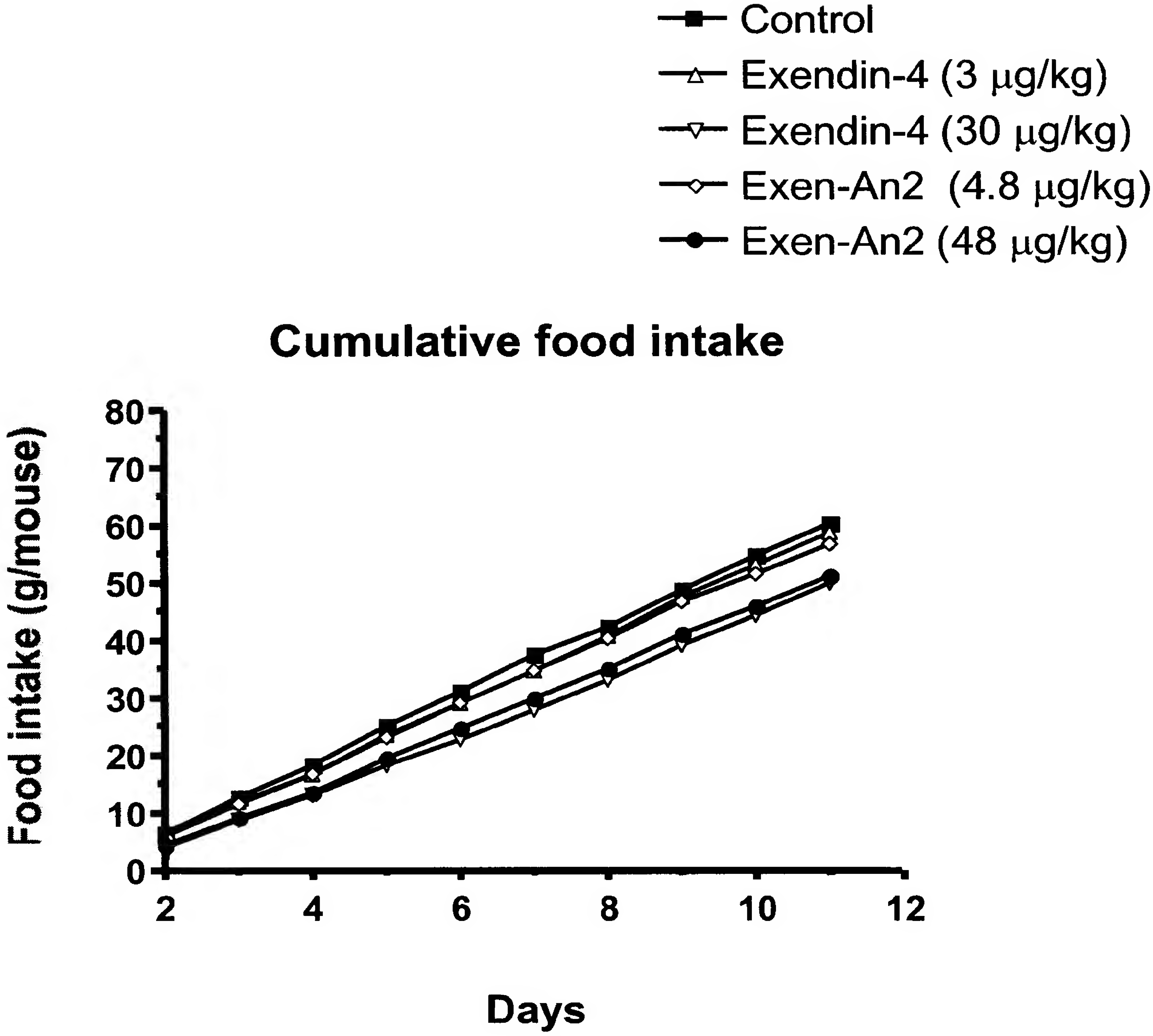

Figure 6

PATENT
ATTORNEY DOCKET NO. V82690WO

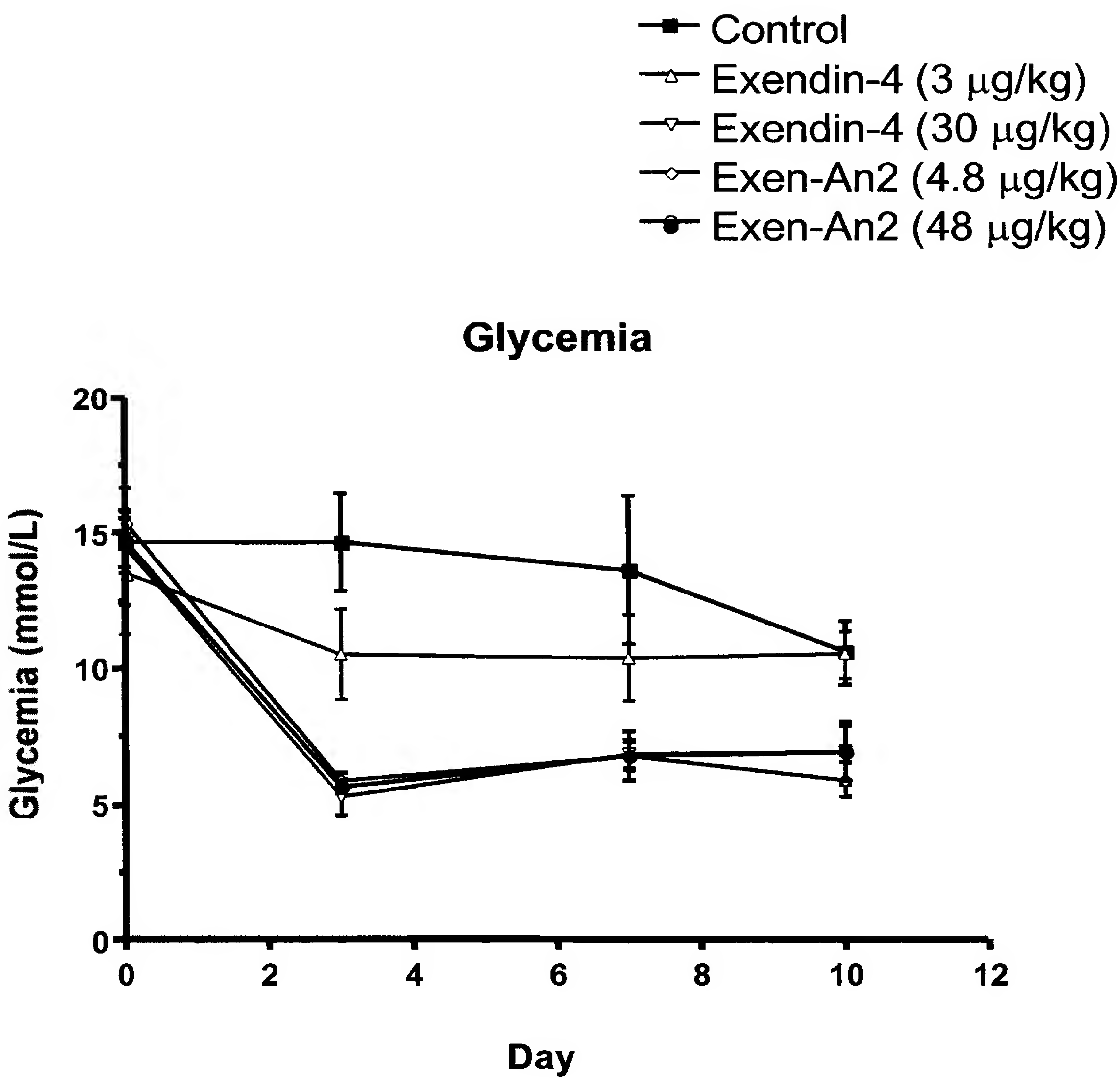

Figure 7

PATENT
ATTORNEY DOCKET NO. V82690WO

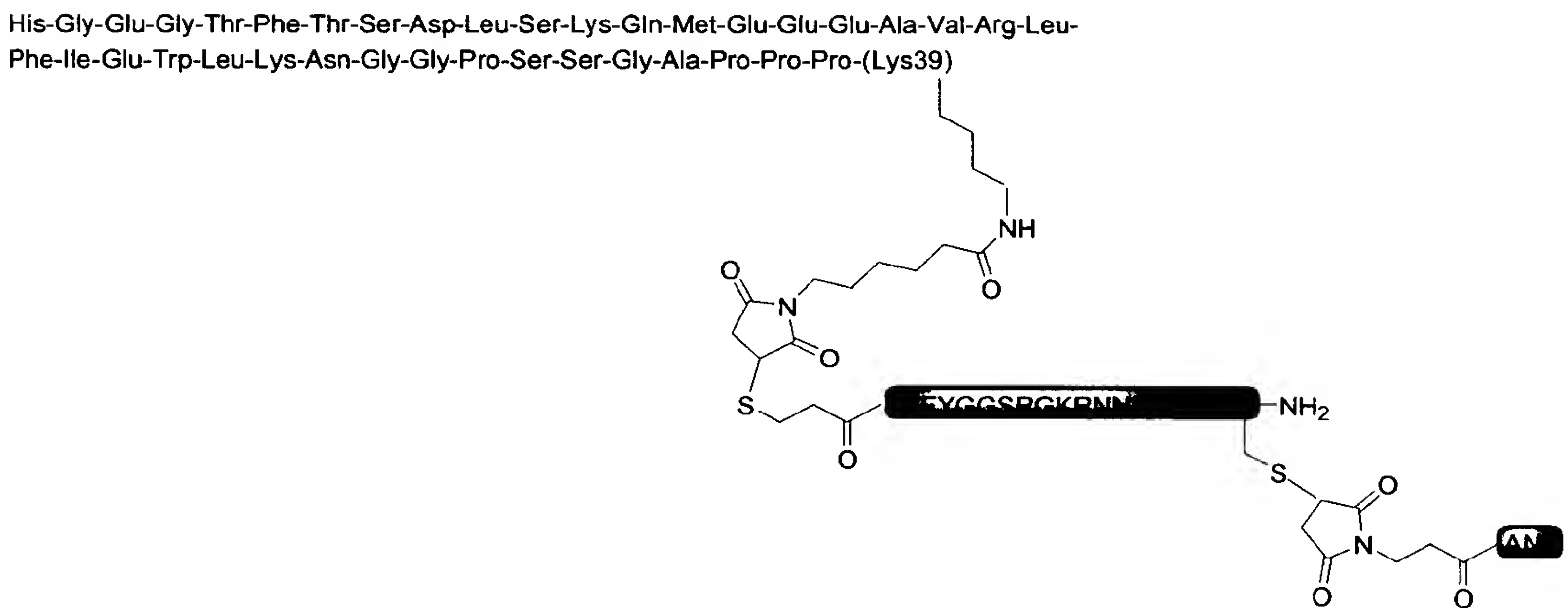

Figure 8A

PATENT
ATTORNEY DOCKET NO. V82690WO

His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-
Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-(Cys32)-Ser-Gly-Ala-Pro-Pro-Pro-Ser

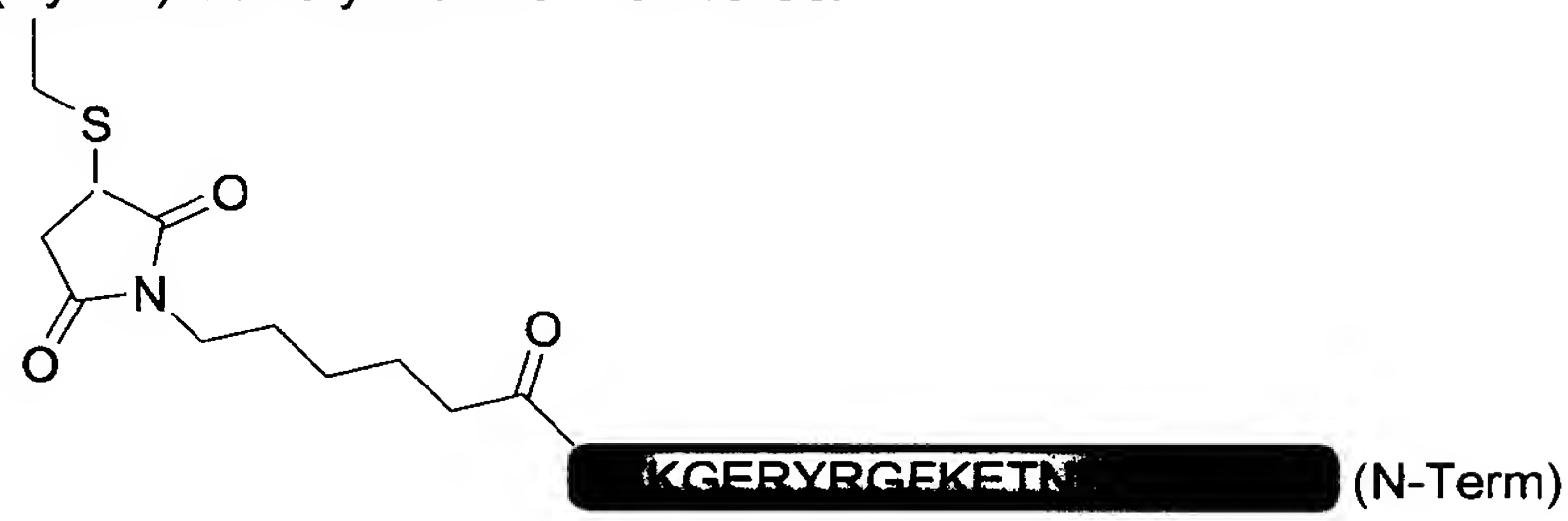

Figure 8B

PATENT
ATTORNEY DOCKET NO. V82690WO

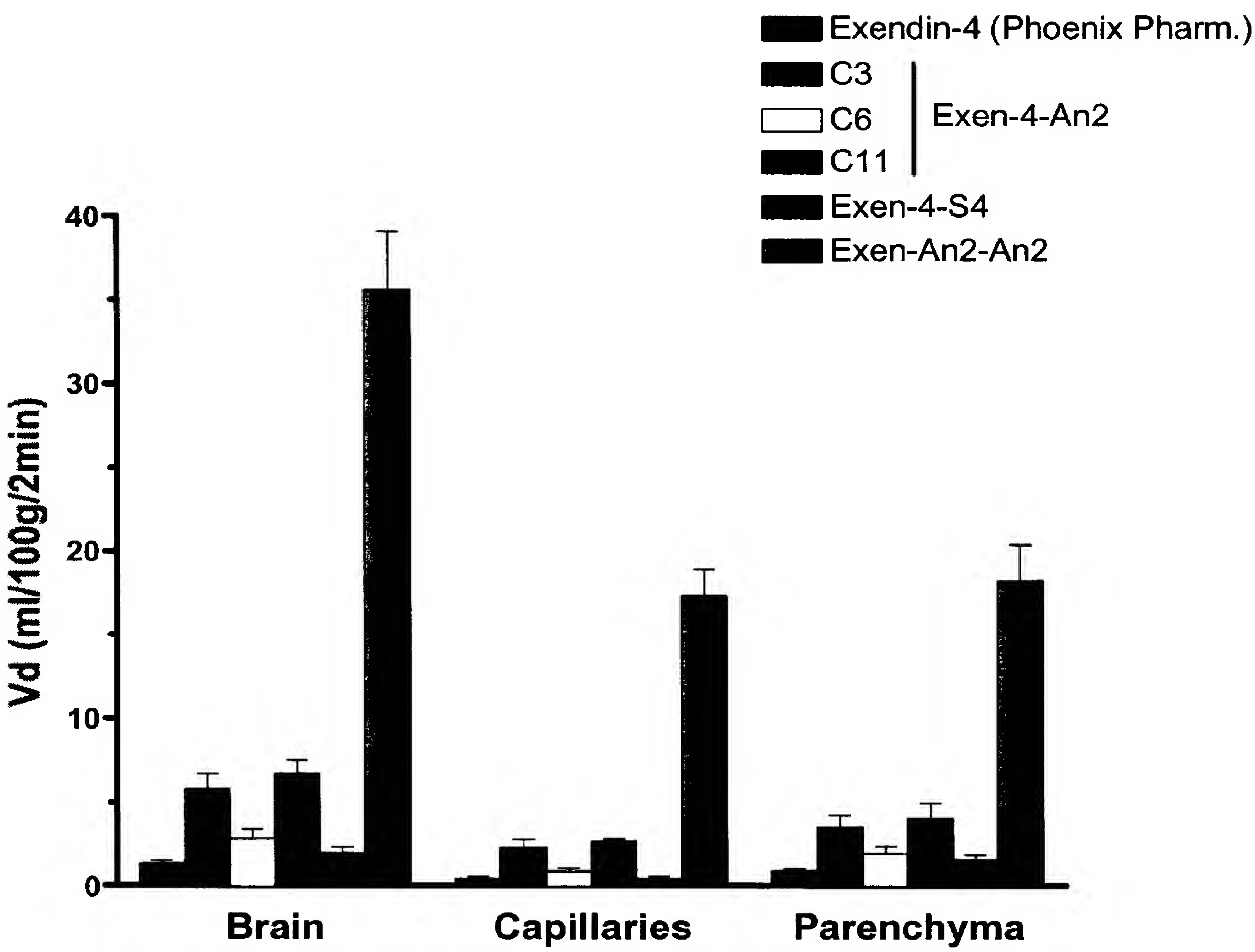

Figure 9

PATENT
ATTORNEY DOCKET NO. V82690WO

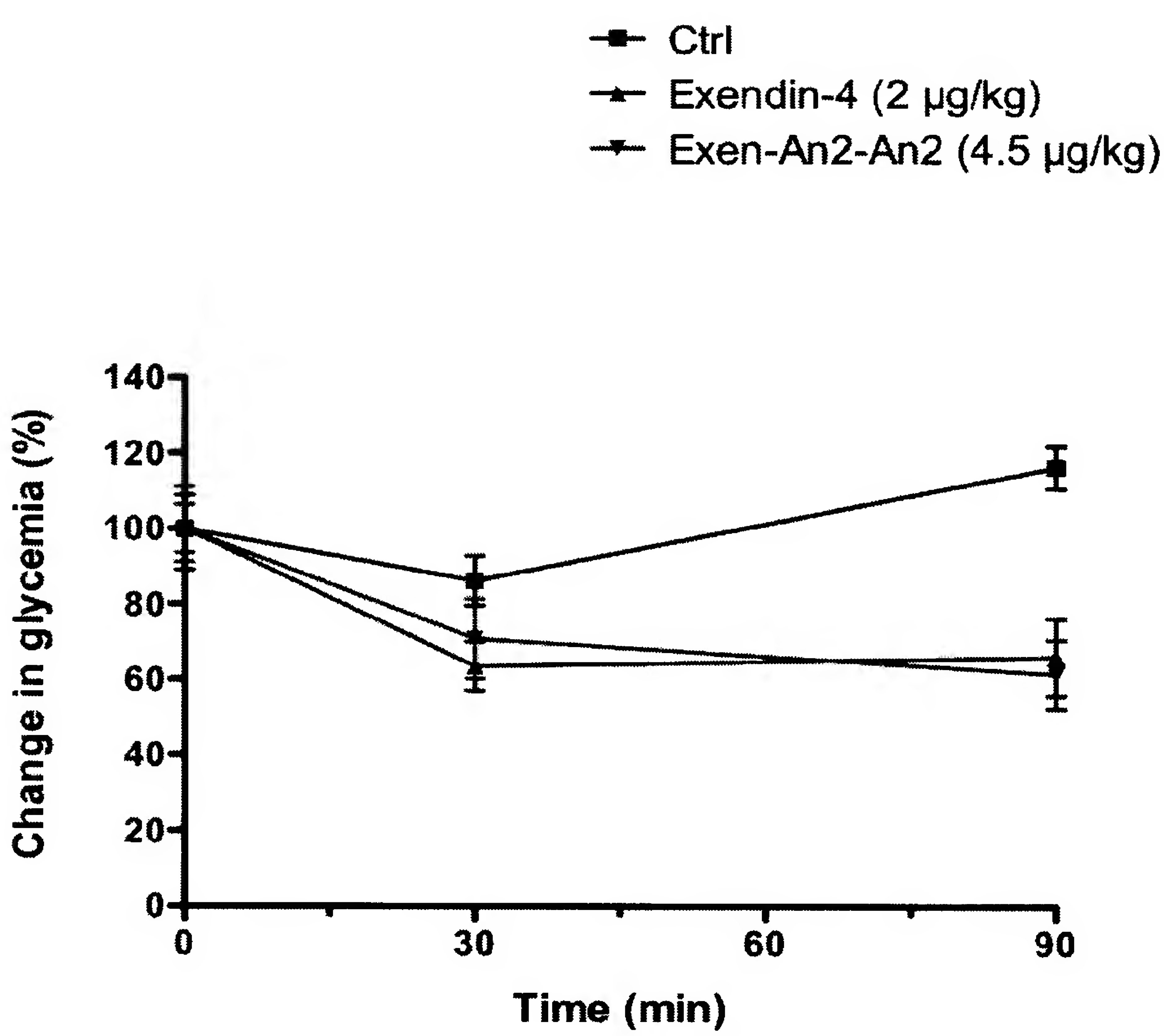

Figure 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001476

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 19/00*** (2006.01) , ***A61K 38/22*** (2006.01) , ***A61K 38/26*** (2006.01) , ***A61K 47/48*** (2006.01) , ***A61P 3/10*** (2006.01) , ***C07K 14/575*** (2006.01) , ***C07K 14/65*** (2006.01) , ***C07K 14/81*** (2006.01) , ***C12N 15/62*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***C07K 19/00*** (2006.01) , ***A61K 38/22*** (2006.01) , ***A61K 38/26*** (2006.01) , ***A61K 47/48*** (2006.01) , ***A61P 3/10*** (2006.01) , ***C07K 14/575*** (2006.01) , ***C07K 14/65*** (2006.01) , ***C07K 14/81*** (2006.01) , ***C12N 15/62*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion, Scopus, Genomequest, Canadian Patent Database. Glucagon-like peptide-1, GLP-1, agonist, exendin, angiopep, aprotinin, diabetes, obesity, blood brain barrier, neurogenesis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2008/012629 A2 (SADEGHI, H. et al.) 31 January 2008 (31-01-2008) pages 2-4; page 11, lines 36-38; examples 1-5	1, 10-12, 17-23, 26-32, 37, 38 33-36
Y	HARKAVYI, A. ct al. "Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease." JOURNAL OF NEUROINFLAMMATION 21 May 2008 (21-05-2008) 5:19 whole document	33-36

[X] Further documents are listed in the continuation of Box [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2009 (22-12-2009)	26 January 2010 (26-01-2010)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Mary Murphy 819-994-4066

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 23-38
   because they relate to subject matter not required to be searched by this Authority, namely :

   Claims 23-38 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-18.

2. [X] Claim Nos. : 9
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

   Claim 9 contains a drawing of a structure wherein the text is obscured by shading. Consequently, the structure that comprises the claimed compound cannot be determined and the subject matter of the claim cannot be searched.

3. [ ] Claim Nos. :
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
A	WO 2006/086870 A1 (BELIVEAU, R. et al.) 24 August 2006 (24-08-2006) whole document	1-8, 10-38
A	DEMEULE M. et al. "Identification and design of peptides as a new drug delivery system for the brain." THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Electronic publication 21 December 2007 (21-12-2007) 324(3):1064-72 whole document	1-8, 10-38

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2008012629A2	31-01-2008	AU 2007278994A1	31-01-2008
		CA 2658654A1	31-01-2008
		CL 21572007A1	01-02-2008
		CN 101511868A	19-08-2009
		EP 2046826A2	15-04-2009
		KR 20090033906A	06-04-2009
		MX 2009001066A	05-02-2009
		NO 20090830A	02-03-2009
		PA 8738401A1	09-02-2009
		US 2009239795A1	24-09-2009
		UY 30497A1	29-02-2008
		WO 2008012629A3	08-05-2008
WO 2006086870A1	24-08-2006	AU 2005327497A1	24-08-2006
		AU 2006272405A1	25-01-2007
		BR PI0520032A2	14-04-2009
		CA 2597958A1	24-08-2006
		CA 2614687A1	25-01-2007
		CN 101160403A	09-04-2008
		CN 101262890A	10-09-2008
		EP 1859041A1	28-11-2007
		EP 1859041A4	08-10-2008
		EP 1907009A1	09-04-2008
		EP 1907009A4	02-09-2009
		JP 2008529539T	07-08-2008
		JP 2009500431T	08-01-2009
		MX 2007010113A	07-12-2007
		RU 2007134566A	27-03-2009
		RU 2008105677A	20-08-2009
		US 7557182B2	07-07-2009
		US 2006189515A1	24-08-2006
		US 2008299039A1	04-12-2008
		US 2009016959A1	15-01-2009
		US 2009082277A1	26-03-2009
		WO 2007009229A1	25-01-2007
		WO 2008144919A1	04-12-2008
		ZA 200706917A	29-10-2008